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THE DISTRIBUTION OF THE BRONCHIAL TREE IN THE BOVINE LUNG

By

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INTRODUCTION

In this communication it is proposed to give a short account of certain aspects of the anatomy of the bronchial system of the normal bovine lung. The distribution of the bronchial tree is neglected in anatomical text books and it is possible that it is of importance in the explanation of the localisation and subsequent distribution of tuberculous lesions.

EMBRYOLOGY AND COMPARATIVE ANATOMY OF THE LUNG

In mammals the lungs arise as a diverticulum from the ventral aspect of the pharynx. This lung bud bifurcates and the respiratory organs are then represented by a tubular trachea and two lung buds or primary bronchi. In the primitive animals this may be the end of development, when the respiratory system consists of a trachea and two bronchi, each with a simple pulmonary sac. In higher forms, the thorax becomes larger and lung buds grow out in regular series from the two main bronchi. The reptilian lung is still more complex but the lung is nevertheless entirely hyparterial, that is to say, all the bronchi arise from the trachea below the point at which it is crossed by the pulmonary artery. In about 95 per cent. of mammals, however, the right lung differs from the left, in that an eparterial bronchus is present which supplies the apical portion of the right lung. This bronchus arises from the main bronchus (90 per cent. of living mammals) or from the trachea, cranial to the point at which the pulmonary artery crosses the trachea. The reasons for this asymmetry are not very clear, but it is recognised that in the left lung, the ascending branch of the first hyparterial bronchus develops more fully to supply a portion of the lung similar to, but smaller than, that served by the eparterial bronchus on the right. It is suggested that the apical portion of the left lung is smaller than the right because it has a lessened opportunity for pulmonary expansion on the left side, where the thoracic volume is smaller than on the right side, in consequence of the rotation of heart and oesophagus in opposite directions. Thus in the mammal an increased respiratory area is obtained chiefly by an increase in size of the right lung. This new respiratory area is at first supplied by the cranial branch of the first right hyparterial bronchus, which enlarges until it becomes as large as the parent bronchus. It is now only a small step in evolution for the area to be supplied by its own eparterial bronchus.

METHODS OF EXAMINATION OF THE MAIN BRONCHI AND THEIR PULMONARY SEGMENTS

The anatomy of the bronchial tree may be investigated by dissection, by making corrosion casts, by the study of bronchograms, and by the injection of air into bronchi to ascertain their distribution. Most of the results in this article have been obtained by dissection methods while doing detailed pathological examinations of tuberculous lungs, but full use has been made of the injection of air method in normal lungs. In the early stages the lungs were fixed in formalin and blunt dissection was carried out; in the bovine lung it is comparatively simple to dissect out even small lung lobules by this method since the interlobular connective tissue is so plentiful. The results so obtained have been confirmed and amplified further by reconstruction of the bronchial tree from multiple slicing of fixed lungs, using a large ham slicing machine. In all, 60 lungs have been examined.

BRONCHIAL TREE OF THE BOVINE LUNG

The larger subdivisions of the bronchial tree are relatively constant and it is the main lobar bronchi along with their primary branches which are described below.

The trachea gives rise to two primary hyparterial bronchi which extend down to the most caudal part of each diaphragmatic lobe of lung. Along their length, the primary bronchi give rise to numerous branches which, with the exception of the first branch on either side, supply the diaphragmatic lobes. On the right side the trachea has the extra eparterial bronchus. The terms primary, secondary, tertiary, etc., as applied to bronchi by some authors, tend to be misleading, since the main bronchus to each diaphragmatic lobe and to the right apical lobe is part of the primary stem and should, therefore, be called a primary bronchus. The main bronchi to the other lobes (left apical, left cardiac, right cardiac and intermediate) however, although analogous to the main diaphragmatic and right apical bronchi, are in fact branches of the primary bronchi and must of necessity be called secondary and their branches tertiary. For this reason these terms will not be used in this communication; the bronchi will be named according to the portion of lung supplied.

Glass (1934) gives the conception of the "broncho-pulmonary segment" defined as the area of lung tissue supplied by a given bronchus. This, however, is an arbitrary conception and the size of any segment depends upon the size of the bronchus which supplies it; thus a whole lobe may be regarded as a broncho-pulmonary segment supplied by a lobar bronchus, while at the other extreme, a terminal lobule may be looked upon as a broncho-pulmonary segment supplied by a terminal bronchiole. When the term broncho-pulmonary segment is used in this article it denotes a portion of lung supplied by one of the lobar bronchi to be described. In the description and diagrams, the broncho-pulmonary segments of each

lobe will be lettered in alphabetical order according to the order of origin from the main lobe bronchus of their associated bronchi. These bronchi will be nominated with the same letter as that given to their pulmonary segments. The diagrams show the surface markings of these broncho-pulmonary segments from two viewpoints, so that every surface can be seen.

ANATOMICAL DESCRIPTION

Right apical lobe. (Figs. 1 and 2). The eparterial bronchus arises from the right lateral surface of the trachea about 10 cm. from its bifurcation. It is about 1.5 cm. in length and then bifurcates into a cranial branch supplying the cranial division of the apical lobe, and a caudal branch supplying the caudal division of the apical lobe.

Cranial Division of the eparterial bronchus to the right apical lobe. This branch (A) is directed ventrally and slightly cranially; it runs down the caudal border of the lobe to its rather bulbous tip which lies ventrally upon the sternum. It has two main and well marked cranial branches. The first (B) is given off near the origin of the stem bronchus and passes cranially and slightly ventrally to supply the dorsal part of the lobe. The second and more distal branch (C) is only slightly smaller than the main bronchus and it also passes ventrally and slightly cranially. Finally, a third and much smaller medial branch (D) supplies the medial portion of the bulbous tip of the lobe. In this way, the anterior lobe is divided into four main broncho-pulmonary segments, three (B, C and A) being demarcated on both the lateral and medial surfaces of the lobe, while the segment (D) is present only on the medial surface.

Caudal Division of the eparterial bronchus to the right apical lobe. This bronchus (A) runs in a ventral and slightly caudal direction to the most ventral tip of the lobe. At the hilum of the lobe, this bronchus gives off a large branch (B) which runs in a caudal direction to supply the most dorsal part of the caudal portion of the right apical lobe. More distally, second and third branches arise, the more proximal (C) of the two running in a cranial and ventral direction, while the other (D) passes in a caudal and ventral direction. The lobe is composed of four broncho-pulmonary segments (A, B, C and D) all being demarcated on both the lateral and medial surfaces of the lobe.

Right cardiac lobe. (Figs. 1 and 2). This lobe is triangular in cross section, with one surface facing laterally and two surfaces meeting at a peak medially. The lobe is supplied by the first branch of the right main stem bronchus and arises from the lateral surface of this bronchus soon after its bifurcation from the trachea. The bronchus (A) runs in a ventral direction to the tip of the lobe, at first medial and superficial but gradually becoming deep in the lung substance. The branches of this right cardiac bronchus are numerous. The

first (B) is very short and quickly bifurcates into two, one running cranially and ventrally and the other running caudally and ventrally. The second (C) runs in a ventral and medial direction to supply the proximal portion of the medial peak of the lobe. The third (D) almost immediately divides into two, in a similar manner to the first, although the two branches may arise separately from the main lobe bronchus. The fourth (E) is similar to the second and runs in a ventral and medial direction to supply the distal portion of the medial peak of the lobe. The last four branches (F, G, H and I) all arise separately and alternately run caudally and ventrally and cranially and ventrally. The lobe in this way is divided into numerous broncho-pulmonary segments, all of which, except the two medial peak segments (C and E) can be demarcated on both lateral and medial surfaces of the lobe.

Right diaphragmatic lobe. (Figs. 1 and 2). This lobe is by far the largest lobe in the bovine lung. It can be represented as being a triangular pyramid with three borders, three surfaces and a base. The mediastinal surface, bounded by the costo-vertebral and mediastinal borders, has an elongated triangular shape, its base being cranial and its apex caudal. This surface closely follows the curve of the diaphragm in a caudal and dorsal direction, so that the apex of the surface becomes confluent with the apex of the other two surfaces to form a peak which fits into the angle where diaphragm, vertebral column and ribs meet, at about the 11th thoracic vertebra. The diaphragmatic surface, bounded by the mediastinal and costo-phrenic borders, is closely moulded to the curve of the diaphragm so that it runs in a caudal and dorsal direction to help form the lobe peak in the vertebral costal diaphragmatic angle, while laterally the surface is curved concavely and runs in a ventro-lateral direction. The costal surface, bounded by the costo-vertebral and costo-phrenic borders, due to the influence of the diaphragm on the shape of the thoracic cavity, is roughly triangular. The apex of the triangle is dorsal and caudal and forms part of the lobe peak. The base of the lobe pyramid is small and is intimately attached to the apical lobes. It can be seen, therefore, that the mass of lung substance is towards the mediastinal surface and since this surface is also triangular with its apex caudal, it follows that the greater portion of lung substance in this lobe is medial and cranial.

The bronchus to this lobe is the direct continuation of the right bifurcation of the trachea. It enters the lung substance at the hilum and passes directly to the lung peak by running in a caudal and dorsal direction parallel, but lateral, to the mediastinal surface; it runs through the thickest portion of the lobe substance. This bronchus can be termed the right caudal bronchus (A). From this bronchus, numerous branch bronchi arise which gradually decrease in size as the apex of the lobe is reached. In addition to this, the origins of these branches become more closely associated towards the apex so that a given length of caudal bronchus gives origin to more

numerous but smaller bronchi caudally, than it does cranially. The general plan of the distribution of these branch bronchi is simple, in that the thick medial portion of the lobe is supplied by a series of relatively short bronchi, five of which pass dorsally, and six to eight ventrally. The numbers vary slightly due to the fact that the caudal ventral branches occasionally arise from common trunks, instead of independently. The remaining lateral portion of lobe substance is supplied by longer and larger lateral branches.

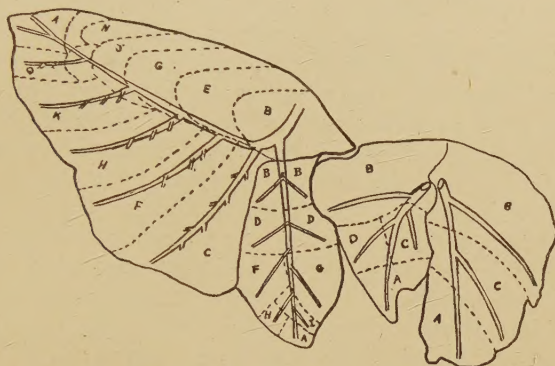


FIG. 1.—Distribution of the bronchial tree in right lung. (Dorsal.)

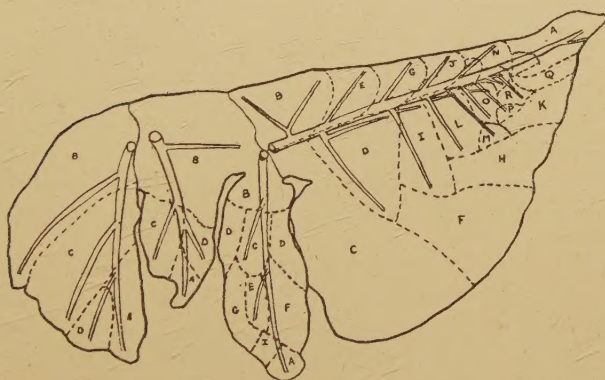


FIG. 2.—Distribution of the bronchial tree in right lung. (Ventral.)

The demarcation of the broncho-pulmonary segments supplied by these various branch bronchi is also relatively simple. The corresponding segments of lung associated with the dorsal bronchi (B, E, G, J and N) are demarcated by bands passing from the ventral border of the mediastinal surface over this surface and then over the dorsal border on to the costal surface of the lobe. The segments of lung supplied by the ventral bronchi (D, I, L, M, O, R and P) can be seen only on the diaphragmatic surface of the lobe and are

directly associated with the dorsal broncho-pulmonary segments at the ventral border of the mediastinal surface. The broncho-pulmonary segments of the lateral branch bronchi (C, F, H, K and Q) occupy the remaining portion of lung surface and are demarcated on both the costal and diaphragmatic surfaces. A brief account of the course of these various branches follows but at the apex end of the lobe, as already mentioned, the origin of the various branches are closely related and are not constant, so that slight variations from the positions indicated in the diagrams will occur.

The first dorsal branch (B) is large and quickly divides into two parts, one of which passes caudally and the other cranially. A smaller branch is given off immediately after the origin of the bronchus and passes in a ventral direction. The 2nd, 3rd, 4th and 5th (E, G, J and N) dorsal branches are all similar, and pass in a dorsal caudal direction, the direction becoming increasingly more caudal and less dorsal as the lung apex is reached. The first three ventral branches (D, I and L) of the caudal bronchus almost immediately after their origin divide into two, one branch passing to supply the cranial part of its segment, while the other runs caudally to supply the rest of the segment. The other ventral branches (M, O, P and R) are inconstant; they may be similar to the above, or the lateral and caudal subdivisions may arise separately. The first lateral bronchus (C) is long and terminates at the costo-phrenic angle supplying the most ventral part of the whole lung. It runs along the caudal border of its lung segment and gives off several branches which run cranially and ventrally to supply the cranial portion of the lung segment. The 2nd, 3rd, 4th and 5th (F, H, K and Q) branches are very similar, except that they become decreasingly shorter and at the same time begin to run more caudally and less ventrally; all, however, terminate in the costo-phrenic border. The 5th lateral (Q) branch is very short and along with the terminal bifurcation of the caudal bronchus supplies the lobe peak in the costo-diaphragmatic vertebral angle.

Intermediate lobe. The bronchus to this lobe is the second branch of the right caudal bronchus and runs to the tip of the lobe. A short distance from its origin cranial and caudal branches arise which supply the cranial and caudal parts of the lobe respectively.

Left apical lobe. (Figs. 3 and 4). This lobe, unlike the right apical lobe, is undivided and is supplied by the cranial division of the first branch of the left bronchus. This first branch originates just caudal to the bifurcation of the trachea and immediately before the left caudal bronchus enters the diaphragmatic lobe. The left apical lobe bronchus (A) passes in a cranial and slightly ventral direction to the apex of the lobe. The first two branches of this lobe bronchus are small; one (B) runs caudally and the other (C) dorsally to supply the base of the lobe. Immediately distal to this first branch, the lobe bronchus gives origin to its two main branches. These two branches arise together, one (D) passing cranially and dorsally and the other

(E) cranially and ventrally. In this way, the lobe is divided into five broncho-pulmonary segments, dorsal (D), middle (A), ventral (E), and two caudal (B and C), in position. The three main pulmonary segments (A, D and E) are demarcated on both the lateral and medial surfaces of the lobe but the caudal segments are demarcated, one (B) on the lateral and one (C) on the medial surface.

Left cardiac lobe. (Figs. 3 and 4). The bronchus to this lobe arises from the common trunk which also gives rise to the left apical bronchus already described. It (A) passes ventrally to the tip of

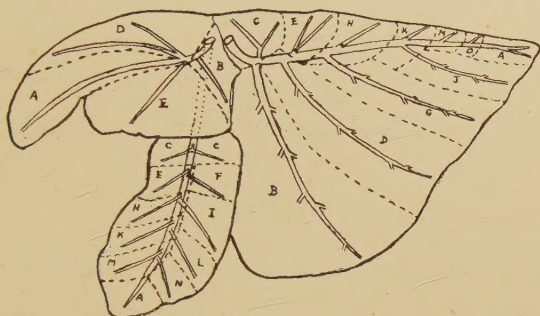


FIG. 3 —Distribution of the bronchial tree in left lung. (Dorsal.)

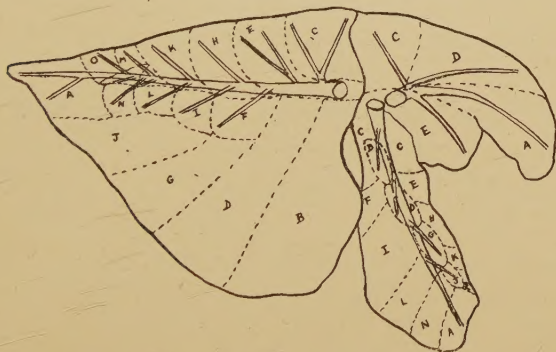


FIG. 4.—Distribution of the bronchial tree in left lung. (Ventral.)

the cardiac lobe as the main stem bronchus. The left cardiac lobe is divided into horizontal segments, five in number (C, E and F, H and I, K and L, M and N), which are supplied by pairs of small cranial and caudal branches, each two originating together from the main stem bronchus. These segments are demarcated on the lateral and medial surfaces of the lobe. In addition on the medial surface of the lobe are four segments (B, D, G and J) which make up the peak of the lobe, since the latter is triangular in cross section with a medial apex. These segments are supplied by small medial branches of the lobe bronchus.

Left diaphragmatic lobe. (Figs. 3 and 4). This lobe has a similar basic broncho-pulmonary structure to that of the right diaphragmatic lobe but varies in detail. There are six dorsal branches (C, E, H, K, M and O) of which both the first and second quickly bifurcate. The ventral branches (F, I, L and N) are smaller and less complex and fewer in number and supply smaller broncho-pulmonary segments than those of the right side. The first ventral branch of the right diaphragmatic lobe is not represented and the corresponding lung segment is incorporated into the 2nd lateral broncho-pulmonary segment. There are only four lateral bronchi (B, D, G and J) but their distribution is similar to those of the other lung.

SUMMARY

The anatomy of the bronchial system of 60 normal bovine lungs is described.

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REFERENCE

Glass, A. (1934). *Amer. J. Roentgenol*, **31**, 328.

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BOVINE PULMONARY TUBERCULOSIS*

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INTRODUCTION

THE present investigation appeared to be warranted in view of the great difference of opinion by many workers as regards the common portal of entry of the tubercle bacillus into the bovine body.

A survey of veterinary literature shows that it was well appreciated by observers at the end of the last century that in cattle bronchial-mediastinal lymph gland tuberculosis was the most frequently occurring form and that such lesions were commonly not associated with lung lesions (M'Fadyean, 1891; Friedberger and Frohner, 1895; Nocard, 1895; Laithwood, 1896; M'Fadyean, 1898). This frequency of bronchial-mediastinal lymph gland tuberculosis in bovines has been fully supported in more recent literature (Stamp and Wilson, 1946; Spartz, 1934). At that time it was not doubted that these lesions arose by inhalation infection. The view that such lesions are produced, almost invariably, by tubercle bacilli entering the body through the mucous membrane of the alimentary tract was later put forward by Von Behring (1903) and by Calmette and Guérin (1905, 1906) and it is now widely stated that the common site of infection leading to bronchial-mediastinal lymph gland tuberculosis is the intestine (Mettam, 1906; Muller, 1906; Schroeder and Cotton, 1906; Cadeac, 1909; Edit., 1908; Edit., 1910; Edwards, 1937; Innes, 1937; Udall, 1943). That the question of the intestinal origin of pulmonary tuberculosis is still unsettled is shown by the experimental work of White and Minett (1941) and Edwards (1937), the viewpoints of these workers being ably reviewed by Glover (1941) who considers that their evidence, like the evidence of much previous experimental work, is inconclusive and that the problem is still a very open one.

It has never been seriously debated that the primary site of infection is intestinal when the only or oldest lesions present in the body are in the mesenteric lymph glands, nor has it been suggested that similar lesions present in the retropharyngeal lymph glands are not indicative of pharyngeal infection. Theoretically, the bronchial-mediastinal lymph glands can become the seat of tuberculous disease by lymphatic drainage of lung infections either aerogenous or haematogenous in origin, or by lymphatic flow from a distant site of infection such as the alimentary tract.

In consequence of this vexed question of alimentary *versus* pulmonary origin of bronchial-mediastinal gland tuberculosis, it was thought that a detailed morbid anatomical examination, along with careful histological examination of cases of bovine tuberculosis, might give some evidence to support one or other theory. It was

* This work was made possible by an Agricultural Research Council Grant held by the Pathology Department, Royal (Dick) Veterinary College, Edinburgh.

considered that despite frequent statements to the contrary (Law, 1912; Wallis Hoare, 1913; Calmette, 1923; Yamagiwa and Jasaki, 1930), it might be possible to demonstrate some lesion in the lung which could be regarded as primary to the bronchial-mediastinal lymph gland lesions and due to aerogenous infection. Such lesions have been extensively described, both in experimental tuberculosis and in spontaneous tuberculosis in the human. In addition, it was thought that an extensive morbid anatomical investigation of bovine tuberculosis might indicate the routes of dissemination of the disease in the body.

METHODS

Material from 100 cases was obtained from Edinburgh abattoir, and no history was available. The animals which were selected from a larger investigation, were those in which, on initial examination for meat inspection purposes, lesions of tuberculosis or suspected tuberculosis were confined to the thorax. Such a selection is justified since the returns from such abattoir inspections have been the foundation of the many statements as to the frequency of thoracic tuberculosis in the bovine.

The following *post-mortem* examination was carried out on all cases. At the abattoir there was a careful examination of the carcass and carcass lymph glands. The lungs and heart, alimentary canal and head, along with the udder and uterus if present, were taken for detailed macroscopical examination at the laboratory, and for histological examination.

Alimentary canal. This was stripped from the mesentery, opened and washed from end to end. The mucosa was then examined for any abnormality, and associated mesenteric and gastric lymph glands cut into multiple thin slices.

Liver. The hepatic lymph glands were examined by thin slicing while the liver substance was examined by slicing on a large ham machine.

Head. The retropharyngeal, parotid and submaxillary lymph glands, mucosa of the mouth, nose and pharynx, and the tonsils were examined.

Lungs and Heart. These were first infused with 2 per cent. formal saline through the pulmonary artery. The whole organs were then immersed in a large bin of formalin for several days until fixed. The regional lymph glands were first examined along with the thoracic duct or ducts. The left bronchial, right cranial bronchial, the right caudal bronchial (rarely present), the caudal mediastinal, middle mediastinal and cranial mediastinal lymph glands were all carefully examined by multiple slicing with a razor. The lung was next dissected into its various lobes, right diaphragmatic, accessory, right cardiac, right cranial and caudal apicals, left apical, left cardiac and left diaphragmatic lobes. Each lobe was sliced into thin slices on the ham machine. All the lobes except the diaphragmatic ones were cut at right angles to the main lobe bronchus, starting at the lobe hilum. The diaphragmatic lobes were sliced parallel to the main lobe bronchus beginning at the mediastinal surface. In this way it was relatively simple

to distinguish the major bronchial pulmonary segments and to record the distribution of lesions within these segments. Later in the investigation, the infusion of the lungs and heart was dispensed with and the tissues were hardened for slicing by freezing in a mixture of salt and ice.

All lesions found were carefully recorded on charts as regards situation, appearance and age.

ANATOMICAL CONSIDERATION

Before describing the observations on thoracic tuberculosis, it is convenient here to review briefly the anatomy of the bovine lung and the lymphatic system of the lung and thorax in so far as it is important to the understanding of the path of infection in tuberculosis. The bovine lungs are divided into lobes by deep interlobar fissures. The left lung is divided into three lobes named apical, cardiac and diaphragmatic. The right lung may be regarded as having five lobes, cranial and caudal apical, cardiac, intermediate and diaphragmatic. The individual lobes themselves can be readily subdivided into broncho-pulmonary segments, each segment being supplied by a single bronchus. The main broncho-pulmonary segments have been described elsewhere (Stamp, 1948), and in this communication the lesions of tuberculosis will be related to these divisions of the lung. In addition, since the regional lung glands are the common seat of tuberculous lesions, it is opportune here to discuss briefly the drainage areas of these glands and their afferent lymphatic vessels. The lymphatics of the lungs have been divided into two groups, deep lymphatics draining lung substance, and superficial lymphatics draining pleura and subpleural lung tissue. The two sets communicate freely at the surface, but since valves are present lymph flow between the two is only from lung to pleura (Baum, 1912). The deep pulmonary lymphatics comprise plexuses which accompany the air passages, arteries and veins in the lung to the hilum. The superficial lymphatics pass over the pleural surface to the mediastinum. In general, the drainage paths of the various lobes can be stated, but it must be recognised that variations may occur. The lymph glands of importance in thoracic tuberculosis are cranial mediastinal, middle mediastinal, caudal mediastinal, left bronchial, right cranial bronchial, right caudal bronchial. The cranial mediastinal and right cranial bronchial are frequently fused while the right caudal bronchial is often absent.

Left apical and cardiac lobes. Both superficial and deep lymphatics drain to the left bronchial lymph gland.

Left diaphragmatic. Superficial lymphatics drain to the caudal mediastinal lymph gland except those draining the more cranial portion of the lobe which enter the left bronchial lymph gland. The deep lymphatics run to the hilum of the lung and for the most part go to the left bronchial lymph gland; a small number, however, go to the caudal mediastinal gland. As an exception, some of the deep vessels may drain to the middle mediastinal lymph gland.

Right apical (cranial and caudal parts). Both superficial and deep lymphatics drain chiefly to the right cranial bronchial lymph gland; a few enter the cranial mediastinal lymph gland.

Right cardiac lobe. Superficial vessels drain to the right caudal bronchial, right cranial bronchial and left bronchial. Deep vessels run to the right caudal bronchial and to the middle mediastinal lymph glands. If the right caudal bronchial gland is absent, all run into the middle mediastinal.

Intermediate lobe. Both superficial and deep lymphatics run to the caudal mediastinal and left bronchial lymph glands.

Right diaphragmatic. Subpleural vessels drain chiefly to the caudal mediastinal lymph gland. Deep lymph vessels drain chiefly to the middle mediastinal gland but some cross to the left bronchial lymph gland.

THORACIC TUBERCULOSIS

Cases with primary lung foci

From the 100 cases of bovine tuberculosis with lesions confined to the thorax on abattoir examination, it is considered that primary lung foci were found in 89 cases (89 per cent.) and that these foci were the origin of the thoracic lymph gland tuberculosis. Of the 89 cases in which primary lesions were found, 72 had one focus, six had two foci, five had three foci, five had four foci, and one had five. In all cases both macroscopic and microscopic examination of lung lesions was carried out to assess whether a lesion could be primary to the regional lymph gland changes or not.

Of the remaining eleven cases, lung lesions were not found in three cases, while in eight cases they were so advanced that it was impossible to ascertain a primary focus. It is noteworthy that in one case where no primary lung lesion was present, a large productive tuberculoma of the larynx was the only lesion present in the body except for the caseation of the cranial and middle mediastinal lymph glands.

The distribution of the primary lung foci in the various lobes and their broncho-pulmonary segments

The situation of the various primary foci in the lungs is shown in Diagrams I and II. As can be seen, both right and left lungs are almost equally involved. The diaphragmatic lobes are more frequently the site of primary infection than the other lobes. No lobe of the lung is immune to primary infection.

The anatomical distribution of the tuberculous disease in the bronchial-mediastinal lymph glands with relationship to the primary foci.

Lesions are found in association with the primary lung foci in the bronchial-mediastinal lymph glands, and the relationship is that the lymph glands directly draining the area of lung involved are

affected first. In every case the anatomical appearance of the foci forming the lung-lymph gland complex leaves little doubt that they antedate any other lesions present in the body. The anatomical relationship of the primary foci to the lymph gland lesions is most clearly demonstrated in the 72 cases which show only one primary lung focus. A similar relationship is also seen when primary foci are multiple, but in these cases the lesions in the related glands are obviously more widespread and the association of primary focus to lymph gland change is not so obvious as in the cases with a single primary focus. The following is a brief summary of the findings in the 72 cases of single primary foci. (See Diagrams III and IV.)

Right lung

Right cranial apical foci give rise to lesions in the right cranial bronchial lymph gland.



FIG. 1.—Distribution of the primary lung foci in left lung.



FIG. 2.—Distribution of the primary lung foci in right lung.

Right caudal apical foci give origin to lymph gland lesions in right cranial bronchial, right bronchial (if present) and middle mediastinal lymph gland.

Right cardiac foci drain to left bronchial, right bronchial and middle mediastinal lymph gland.

Right diaphragmatic. Primary foci occurring in the cranial, broncho-pulmonary segments of this lobe give rise to associated lesions in the left bronchial, middle mediastinal and caudal mediastinal lymph glands, while those occurring in the distal broncho-pulmonary segments drain chiefly into the caudal mediastinal lymph gland. There is little doubt that lesions in the cranial broncho-pulmonary segments drain along both superficial and deep lymphatics to the middle mediastinal and left bronchial glands. Primary foci situated in the caudal broncho-pulmonary segments drain almost entirely along the superficial lymphatics to the caudal mediastinal lymph gland, and this route appears to be the most direct path from primary lesions so situated to a regional lymph gland.

Accessory foci drain to both left bronchial and caudal mediastinal lymph glands.

Left lung

Left apical and cardiac lesions drain directly to the left bronchial lymph gland.

Left diaphragmatic foci in the cranial broncho-pulmonary segments drain chiefly to both the left bronchial and caudal mediastinal lymph glands. Primary foci in the caudal broncho-pulmonary segments, as on the right side, drain directly into the caudal mediastinal lymph gland.

It will be seen that if these recorded observations of primary complexes are compared with the normal lymphatic drainage areas, they agree closely.

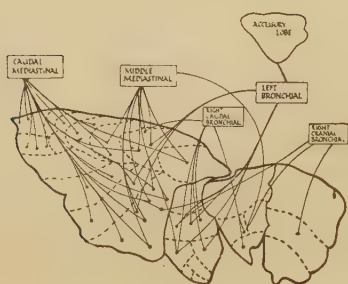


FIG. 3.—Anatomical relationship of single primary lung foci in the right lung to the regional lymph glands.

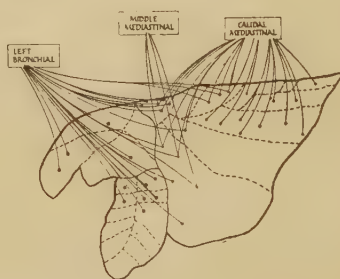


FIG. 4.—Anatomical relationship of single primary lung foci in the left lung to the regional lymph glands.

APPEARANCE AND SIZE OF THE PRIMARY LUNG FOCUS

In accordance with their macroscopic appearance and size, the primary lung foci can be classified as shown in Table I, which also shows the type of lesions found in the associated regional lymph gland or glands. In every case histological examination of lung and lymph gland lesions indicates that the lymph gland lesions are never older than those in the lung.

Encapsulated caseous calcified nodules 1 cm. or less in diameter

Fifteen lesions of this type are found occurring in 13 cases of primary lung infection. They vary in size from $\frac{1}{2}$ to 1 cm., and all are characterised by a definite fibrous tissue capsule surrounding caseous or calcified caseous material. Obvious macroscopic cavitation is occurring in three of these lesions leading to local dissemination of the lesion. The regional lymph gland shows encapsulated caseous nodes of similar age and size to the lung lesions (see Figs. 1 and 2).

Young lesions 2 cm. or less in diameter obviously still progressing, and with no fibrous tissue encapsulation

Forty-six such lesions are described as occurring in 26 cases of primary lung infection, three of these cases having four lung foci each: they vary considerably in appearance. Very young lesions consist of one or several areas of consolidation within a primary lung lobule, spreading peripherally and at the same time undergoing slight central caseation. In this group, however, the majority are at a later stage of development and consist of condensed lung tissue surrounding a focus of recent caseation which vary in size from 2 mm. up to 2 cm. in diameter. Obvious macroscopic cavitation is occurring in 12 of these lesions leading to slight local bronchial dissemination of infection. None show any degree of encapsulation with fibrous tissue, although the older lesions show a pale halo of tuberculous granulation tissue and condensation of surrounding lung tissue. This, however, does not represent healing but merely the progressing edge of an active focus.

The corresponding lymph gland lesions vary in appearance, depending upon the development of the lung focus. They may consist of early small caseous tubercles with or without granulation tissue capsules, or they may have developed into areas of early confluent caseation. In cases where the lung lesion appears to be progressing rapidly, those in the associated lymph gland are comparatively large and consist of areas of tuberculous granulation tissue undergoing diffuse multifocal caseation (see Figs. 3, 4, 5 and 6).

Nodes of coalescing caseous acinar broncho-pneumonia

Fourteen such lesions, varying in size from 2 to 5 cm. in diameter, occur in 13 cases of primary lung infection: they consist of several or many foci of caseous acinar broncho-pneumonia which are coalescing, giving finger-like processes of caseation. They may involve one or many primary lung lobules so that the primary focus may be small or large. The whole lesion is usually encapsulated with a slight fibrous tissue capsule but there is no connective tissue capsule between the individual acinar caseous foci. All stages in the confluence of the individual acinar foci to a caseous abscess with definite surrounding encapsulation of fibrous tissue can be traced. Cavitation of these lesions has occurred in nine cases.

The regional lymph glands are almost completely replaced by cellular granulation tissue showing varying degrees of multifocal caseation (see Figs. 7, 8, 9 and 10).

Encapsulated caseous calcified nodes 1 to 2 cm. in diameter

Eighteen such primary lung lesions are found in 14 cases of pulmonary tuberculosis, consisting essentially of caseous and frequently calcified nodes of tuberculosis all having a definite fibrous tissue capsule. In the younger lesions a multifocal origin is discern-

ible. Cavitation along the associated bronchus occurs frequently and 17 of the 18 described in this category show very evident macroscopic signs of such progression (see Figs. 11, 12, 13 and 14).

Large encapsulated caseous calcified lesions

These lesions are essentially similar to those described in the last section except that they differ considerably in size, varying from 2 to 5 cm. in diameter; they are caseous and calcified and heavily encapsulated with dense fibrous tissue. The origin of these large aggregations from the confluence of several caseous foci is clearly seen in some of the younger lesions; 26 are found in 18 cases of primary lung infection. All the lesions show definite macroscopic evidence of bronchial cavitation.

The lymph gland lesions in both the above categories consist of hyaline encapsulated caseous calcified abscesses of varying size, while occasionally the whole gland is replaced by massive caseation surrounded by a very thickened fibrous tissue capsule (see Figs. 15, 16, 17 and 18).

Fibrotic cavity

One such primary lesion is seen. The cavity is small and heavily encapsulated with fibrous tissue and is drained by a small bronchus. Despite the heavy encapsulation healing is not complete for small active tubercles, in which acid-fast bacilli are present, line the cavity. Local bronchial dissemination has occurred. The associated lymph gland lesion consists of a thick walled caseous abscess (see Fig. 19).

Acute cavitating caseous pneumonia

Only one such primary lesion is seen and in this case a major broncho-pulmonary segment is undergoing massive caseation and acute cavitation. The regional lymph glands are grossly enlarged, soft and caseous with no fibrous tissue reaction (see Fig. 20).

Nodular broncho-pneumonia

Three such lesions are found in three cases of primary lung infection. They vary in size from 2 to 5 cm. in diameter and consist of several neighbouring lung lobules which are undergoing caseation and encapsulation. The juxtaposed lesions appear of similar age. Confluence does not occur owing to the formation of the fibrous tissue capsules between individual nodes. Macroscopic bronchial cavitation is seen in all three cases. The associated lymph gland lesions consist of encapsulated caseous abscesses (see Figs. 21 and 22).

RESULTS

Primary lung lesions

Of a total of 100 cases of thoracic tuberculosis, 89 (89 per cent.) were shown to have recognisable primary lesions in the lung, while

in eight cases the lung disease was too advanced to enable one to recognise the primary focus. In only three cases was a lung lesion not found. In every case careful macroscopic and microscopic examination of both lung and lymph gland lesions was carried out in order to assess the comparative age of the two lesions. For this purpose the general concepts of tuberculosis pathology have been followed. It is clear from these findings that when tuberculous lesions are found confined to the bronchial mediastinal lymph glands, then in nearly every case an associated lesion will be found in the

TABLE I
THE RELATION OF THE PATHOLOGICAL CHANGES IN THE PRIMARY LUNG FOCI
TO THOSE IN THE ASSOCIATED TRACHEO-BRONCHIAL GLANDS

<i>Number of cases</i>	<i>Pathological lesion in lung (primary focus)</i>	<i>Number of cases</i>	<i>Pathological lesion in regional lymph glands</i>
13	Encapsulated caseous calcified nodules, 1 cm. or less in diameter	13	Encapsulated calcified caseous nodes, $\frac{1}{4}$ –2 cm. in diameter
26	Early lesions, obviously still active and with little or no encapsulation	9	Early caseous tubercles
		2	Early confluent caseation
		6	Diffuse multifocal caseation of granulation tissue
		1	Caseation with slight encapsulation
		8	Nodular caseation
14	Encapsulated caseous calcified nodes, 1–2 cm. in size	14	1–3 cm. encapsulated calcified caseous nodes
18	Large encapsulated caseous calcified lesions	17	Large hyaline encapsulated abscess
		1	Massive caseation
1	Acute cavitating caseous pneumonia	1	Enlarged soft and caseous
1	Fibrotic cavity	1	Large encapsulated caseous calcified abscess
13	Coalescing caseous acinar broncho-pneumonia	13	Diffuse caseating productive granulation tissue
3	Nodular broncho-pneumonia	3	Encapsulated caseous calcified abscess

lung substance. In addition, both naked eye and microscopical examination showed that the foci in the lung and in the related lymph glands were of the same age or those in the lymph glands more recent. (See Table I.)

In all 124 primary lung foci were seen. In the majority of cases they were single but in 17 were multiple. In size and appearance and age they varied very considerably. Thus in more than 25 per cent. of cases the primary focus was less than 1 cm. and in several

were only 2 mm. in diameter and were difficult to locate. The remaining primary lesions varied in size from 1 cm. to 5 cm. In appearance and age the primary foci varied from an early cellular infiltration of a lung lobule to a very large heavily encapsulated caseous calcified mass. Four cases had atypical primary foci ; in one

TABLE II
AGE DISTRIBUTION OF PRIMARY LUNG FOCI ACCORDING TO THEIR
MACROSCOPIC CHARACTERS

<i>Character of primary focus</i>		<i>Under 1 year</i>	<i>1-2 years</i>	<i>2-3 years</i>	<i>3-4 years</i>	<i>Over 4 years</i>
Encapsulated caseous calcified nodules, 1 cm. or less in diameter	H	1	1			
	B	6		3	2	
	C	6			1	5
Encapsulated caseous calcified nodes, 1-2 cm. size	H	1	1			
	B	7	1	4	2	
	C	6			2	4
Large encapsulated caseous calcified lesions	H	1	1			
	B	10		4	6	
	C	7			2	5
Fibrotic cavity	H					
	B	1			1	
	C					
Nodular broncho - pneumonia	H					
	B	3			3	
	C					
Total		49	1	4	11	14
Early lesions with little or no encap- sulation	H	1		1		
	B	15		8	7	
	C	10				10
Acute cavitating case- ous pneumonia	H					
	B					
	C	1			1	
Coalescing caseous acinar broncho - pneumonia	H	2		2		
	B	9	3	5	1	
	C	2			1	1
Total		40	3	16	10	11

H = Heifers, 6
B = Bullock, 51
C = Cow, 32

the primary focus consisted of a large, acute, cavitating tuberculous pneumonia, while in the three others it was an area of chronic nodular broncho-pneumonia similar to the lesions seen in post-primary infection. Evidence of marked healing in the form of surrounding fibrosis was seen in 49 cases (55 per cent.). In one case the primary

lesion consisted of a small cavity surrounded by dense fibrous tissue. In only one case was there any evidence of ossification. There was no, or only slight, evidence of healing in 40 cases (45 per cent.). Caseation and calcification in the primary focus was usual except in the very youngest lesions.

Relation of primary lung foci to bronchial mediastinal lymph glands

The relationship between primary lung focus and regional lymph gland was such that the lymph glands in direct anatomical relation to the segment of lung involved were the most grossly diseased, but in many cases the infection had clearly progressed to other lymph glands. In no case was there any obvious chain of foci between lung and gland, so that it would appear that the tubercle bacilli must be carried directly in the lymph flow without causing intermediate lesions.

Dissemination from the primary infection

In the bovine the most common form of dissemination from the primary lesion is bronchogenic arising by softening of the caseous material. Thus, out of a total of 89 cases where the primary lesion has been determined, 61 show evidence of very definite bronchial spread. It was more noticeable, however, that if one excepts the 26 cases where the infection was obviously very recent, then out of the remaining 63 more advanced cases, 49 (80 per cent.) have developed bronchial pneumonia arising from ulceration of the primary lesion. Haematogenous dissemination determined by involvement of peripheral carcase lymph glands, kidney, etc., occurred in only five cases.

DISCUSSION

Portal of infection. The finding of primary lung foci in bovine tuberculosis is not in agreement with many previous statements in the literature of bovine tuberculosis (Friedberger and Frohner, 1895; Law, 1912; Wallis Hoare, 1913; Nocard, 1895; Calmette, 1923; M'Fadyean, 1891; Theives, 1939; M'Fadyean, 1898; Laithwood, 1896; Whipham, 1909; Yamagiwa and Jasikawa, 1930), but is in agreement with Nieberle (1931). From the evidence presented above, it appears unlikely that the lung lesions arise by lymphogenous spread from the lymph glands, nor indeed was there any suggestion that retrograde lymphatic spread back into the lung had occurred at all. Regarding the portal of entry of the tubercle bacilli which cause the primary lung focus, one is therefore left with two theoretical possibilities. Either the bacilli enter the body by the mucous membrane of the digestive tract and do not produce foci until they have been transmitted to the lungs by the blood stream as suggested by Von Behring, or the bacilli enter the lung directly by inhalation. Although the histological features of primary lung lesions in the bovine have not been described in this communication, it is necessary to say that in those cases where the primary focus was at its earliest

stage, it consisted histologically of a small area of tuberculous broncho-pneumonia and, so far as could be seen, there was no evidence to suggest a vascular origin to these lesions. In fact, the lesions appeared to be characteristic of inhalation infection. (It is intended to describe the histological features of primary lung lesions in a later communication.)

Age distribution of primary foci. Nieberle (1931) has divided bovine tuberculosis into calfhood and adult types with differences similar to those frequently described between childhood and adult tuberculosis. Thus in the calf there is gross caseation of the regional lymph nodes associated with a primary lung focus, the whole being commonly called a primary complex (Ranke, 1916) from which frequently metastatic haematogenous lesions disseminate. On the other hand, he states that adult bovine pulmonary tuberculosis is characterised by (a) spread being predominatingly intra-bronchial; (b) absence of macroscopic lesions in the pulmonary lymph nodes; (c) predominance of fibrosis; and (d) cavitation. In the human there would appear to be two possibilities for this division into types: (a) differences due to acquired resistance from a previous infection leaving a healed primary complex; or (b) an age determined native resistance (Rich, 1944). It has, however, previously been shown that bovines are not commonly infected when young but acquire lesions for the first time in adult life (Stamp and Wilson, 1946) so that if such a division into calfhood and adult types as described by Nieberle does in fact occur, then these differences are due not to any acquired resistance but are due to an age determined resistance. The present investigation, however, does not support the contention of Nieberle, for out of a total of 100 animals examined, one was under one year of age, seven were under two years, 27 under three years, 29 under four years and 25 over four years of age, so that practically all the animals were adult; yet 89 per cent. of them showed the presence of caseation of regional lymph nodes associated with a lung focus. It could, of course, be argued that these lesions were formed during calfhood, but surveys of abattoir material do not support this contention, nor does the fact that 40 of the present cases had active and young lesions present. Further evidence against the view that age in itself alters the form of the disease is clearly given in Table II, when it can be seen that the type of the primary pulmonary lesion is in no way influenced by the age of the animal. Thus many young, actively progressing lesions are seen in the older age groups while comparatively static encapsulated lesions are found in the younger groups. Thus, in the bovine, it would appear incorrect to divide tuberculosis into calfhood and adult tuberculosis since such a classification implies that there are anatomical differences in the disease due to differences of immunity caused by age. It must be stressed, however, that it is not doubted that a form of chronic isolated pulmonary tuberculosis does occur in the bovine, but this usually follows primary infection and is

independent of age. This form of pulmonary tuberculosis will be described in a later communication.

Anatomical distribution of primary foci. No portion of the lung parenchyma failed to show primary lesions so that there is no predilection site for its location. Nevertheless, lesions were more frequent in the diaphragmatic lobes even after consideration is given to the greater volume of lung parenchyma in the diaphragmatic lobes (Medlar, 1940). The findings are also in agreement with those of Smith (1894), Medlar (1940) and Nieberle (1931) in that in the diaphragmatic lobes the most common area for lesion location is on the dorsal aspect of the lung. There was no distinct difference in the incidence of primary infection between left and right lungs.

Factors responsible for the characteristics of the primary lung foci in the bovine. It has been stated by Rich (1944) that when a pathologist is faced at autopsy with an established lesion in an organ it would be necessary to know the following factors before any accurate understanding of its development could be made: (a) the virulence of the infecting bacilli; (b) number of bacilli that initiate infection; (c) the degree of native resistance; (d) the degree of acquired resistance present or capable of being developed; (e) degree of hypersensitivity existing or capable of being developed; (f) the tissue in which infection occurs.

In the individual case these factors are usually undetermined, but it is possible to make some estimate of the degree of development of such factors as native resistance, acquired resistance and hypersensitivity in the bovine by observing the lesions in the spontaneous disease in large numbers of cattle. Since the same differences in the reaction of the first infection recur regularly, there can be little doubt that some conclusions can be made. It is thought that the present series might give some information on these aspects of the disease. In the first place, it would appear that the native resistance of the bovine, at least in adult life, is comparatively low for there is little pathological evidence in this series to suggest that the normal bovine body exerts any great restraint upon the progress of the first infection, for in the majority of cases caseation is prominent and healing is inconspicuous while regional lymph nodes frequently become massively involved.

The question of acquired resistance in tuberculosis has been a subject of much controversy, and it is to Ranke (1916) that we owe the widely accepted view that tuberculous infection can be divided into three stages: primary infection, second or hypersensitive stage, and third stage of increased resistance depending upon the development of acquired resistance. It is thought that in the majority of childhood primary infections, acquired resistance develops promptly and effectively, for the lesions are arrested and encapsulated while still very small, giving rise to the familiar non-progressive, globular little lesions of the primary complex (Rich, 1944). In cattle, however, this is not usually the case, for in our series in only 13 cases could

one say that the lesion had been promptly arrested as described above. Much more commonly (46 cases), the primary lesion was comparatively large and caseous, reaching in many cases to 5 or 6 cm. in diameter. It is, therefore, suggested that in cattle the primary focus progresses further than is the rule in the human. Nevertheless, these large caseous lesions do eventually become arrested and encapsulated so that if Ranke's hypothesis is accepted then it would appear that in cattle the second stage of hypersensitivity is prolonged, but that eventually acquired resistance strongly develops and a localisation of the caseous mass begins. It has, however, already been shown that this localisation in the majority of cases is only partially successful, for bronchial dissemination giving rise to a chronic broncho-pneumonia is common. For these reasons the writer considers that the use of the term primary complex should not be used in cattle since it is misleading to those who recognise its pathology in human tuberculosis.

By many, however, the views of Ranke are not accepted as the explanation for the difference between childhood and adult tuberculosis. Krause (1925) believes that the differences are due to (a) difference in the patency of the lymphatics at various ages; (b) difference in lymphoid locations; (c) difference in the ability to form fibrous tissue at different ages, while Rich (1944) suggests that the differences are due not to acquired resistance conferred by a previous immunising infection, but are due to an increase in native resistance resulting in some way from the ageing processes, *i.e.*, there is pathological evidence that the adult reacts to primary infection in a manner different from the child. Rich states, however, that there has been no experimental proof that young and adult animals differ in their reaction to the tubercle bacillus. Most certainly, in the present investigation, the cattle examined varied in age from under one year to nine years old and, as can be seen by Table II, there is no evidence to suggest that age varies the type of reaction to primary infection in the bovine. Most certainly the pathological evidence in bovine tuberculosis is that acquired resistance, following primary infection, plays the major role in altering the type of reaction that occurs in subsequent infection, and that the concepts of Krause and Rich cannot be applied in bovine tuberculosis.

SUMMARY

The anatomical considerations of bovine thoracic tuberculosis are reviewed.

The occurrence and distribution of primary lung lesions in 100 cases of bovine thoracic tuberculosis, and the macroscopic appearances of the primary lung lesions, are described.

The pathway of infection, the dissemination from primary infection and pathogenesis of bovine tuberculosis, are discussed.

The anatomical relationships of lung and lymph gland lesions are described.

FIG. 1

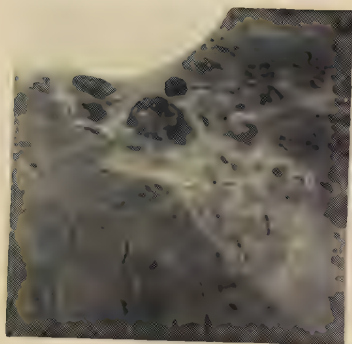


FIG. 2

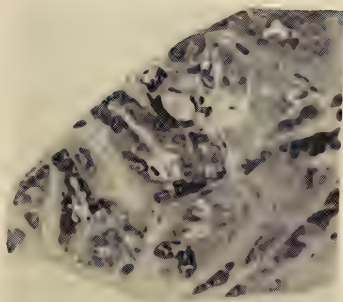


FIG. 3



FIG. 4



FIG. 5



FIG. 6



FIGS. 1 and 2.—Encapsulated caseous calcified primary lung lesions, 1 cm. in diameter.

FIGS. 3, 4, 5 and 6. Young lesions still progressing and with no encapsulation.

FIG. 7

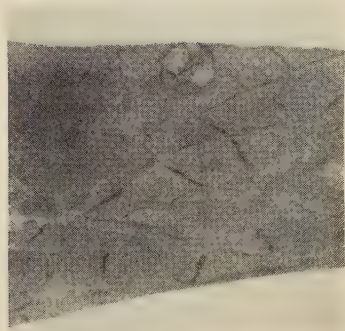


FIG. 8

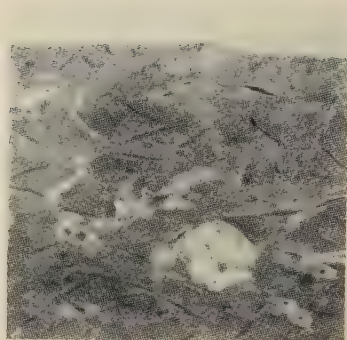


FIG. 9

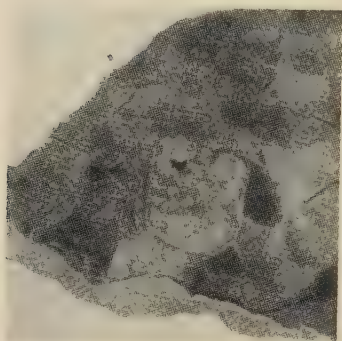


FIG. 10

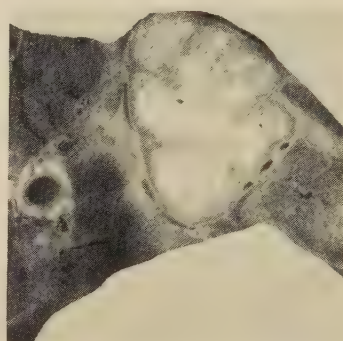


FIG. 11

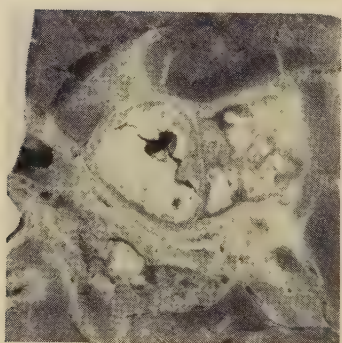


FIG. 12

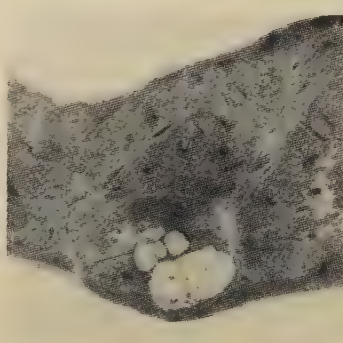


FIG. 13

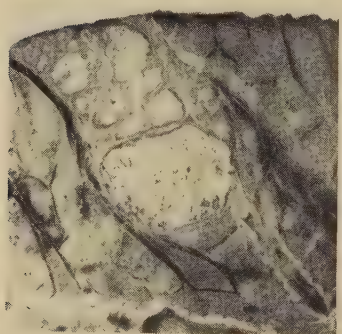
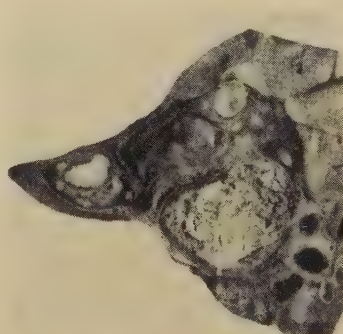


FIG. 14



FIGS. 7, 8, 9 and 10.—Coalescing caseous acinar broncho-pneumonia with encapsulation.
 FIGS. 11, 12, 13 and 14.—Encapsulated caseous calcified nodes, 1 to 2 cm. in

FIG. 15

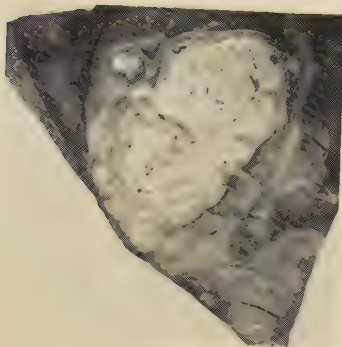


FIG. 16



FIG. 17

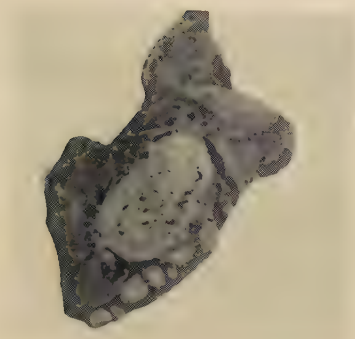


FIG. 18

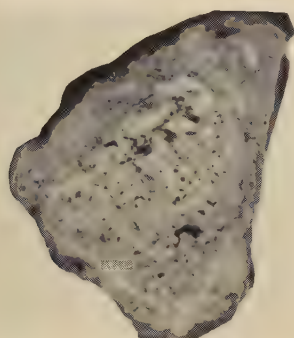


FIG. 19

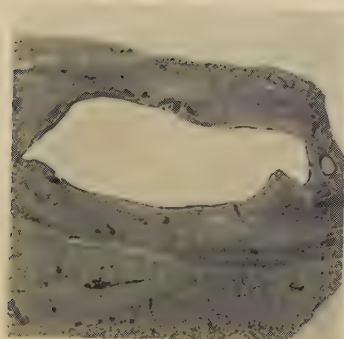


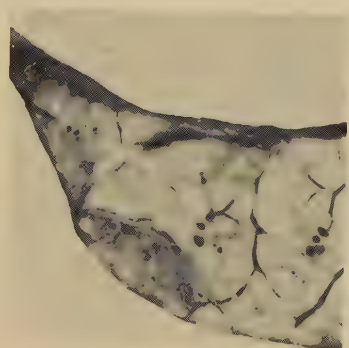
FIG. 20



FIG. 21



FIG. 22



FIGS. 15, 16, 17 and 18.—Large heavily encapsulated caseous calcified lesions.
FIG. 19.—Fibrotic cavity.
FIG. 20.—Acute cavitating caseous pneumonia.

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EXPERIMENTS ON THE PROTECTION OF LAMBS AGAINST TICK PYAEMIA BY THE USE OF STAPHYLOCOCCAL TOXOID

By

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INTRODUCTION

An experiment on the protection of lambs against naturally occurring tick pyaemia in the field was carried out in a tick-infested district in Northern Ireland (Foggie, 1943). In this experiment, of 99 immunised lambs three developed pyaemia, and of 91 untreated lambs 16 developed pyaemia.

Further experiments were planned to show : (a) that a satisfactory immunity against tick pyaemia could be set up in lambs by the injection of staphylococcal toxoid, and (b) that the injection of staphylococcal toxoid in ewes produced a passive immunity in their lambs.

EXPERIMENTS ON THE IMMUNISATION OF LAMBS WITH STAPHYLOCOCCAL TOXOID

METHODS

The lambs used in these experiments were from a flock of cast Blackface ewes bought from a tick-free hill farm and maintained at Moredun Institute.

Both α and $\alpha\beta$ alum-precipitated staphylococcal toxoids were employed as immunising agents. The toxoid was given intramuscularly into the thigh in doses of from 1 to 10 c.c. Apart from transient stiffness immediately after the injection, no ill-effects attributable to the toxoid were observed. The α and β staphylococcal anti-haemolysin contents of the blood of each lamb were tested at intervals throughout the course of the experiments. The results of these tests are given in International Standard Units.

The resistance of the lambs to staphylococcal infection was tested by injecting a suspension of staphylococci into the jugular vein. An $\alpha\beta$ haemolytic staphylococcus (strain 1468) originally isolated from the mouth of a ewe was used to set up infection. This strain was seeded into tubes of Worth's medium which were stored at room temperature. To make up the infective dose, five drops of the Worth's medium culture were spread over a Hartley's agar slope and incubated for 18 hours at 37°C. The growth was washed off with normal saline and a suspension equal in density to tube No. 8 on the Brown's scale was made up. The infective dose was 1 c.c. of a suitable dilution of this suspension.

RESULTS

In 1945, 55 lambs were available for experiment, 16 being used to find the minimum infective dose of staphylococci for lambs of 17 days of age. In the experiment as originally planned the remaining 39 lambs were to be divided into three groups. Group 1 was to receive three injections of α toxoid from the 10th to the 14th day, Group 2 three injections of $\alpha\beta$ toxoid from the 10th to the 14th day and Group 3 was to be an untreated control group.

It soon became apparent that lambs of this age would not respond to the injection of toxoid by the formation of anti-haemolysins. The experiment was, therefore, discontinued and the remaining lambs were used in an attempt to determine the age at which a response to toxoid could first be obtained. They were divided into groups each of which was treated with toxoid at a different age level.

In these experiments the number and size of the doses of toxoid and the types of toxoid used were varied in an attempt to find an optimum antigen for the production of anti-haemolysin. No infor-

TABLE I
ADMINISTRATION OF TOXOID TO LAMBS OF DIFFERENT AGES

Age of lambs at injections of toxoid	Number of lambs in group	Number of lambs with final titres of	
		1 to 9 units	Over 9 units
10 to 14 days	6	0	0
14 " 19 "	4	0	0
20 " 24 "	4	0	0
25 " 28 "	7	4	0
27 " 30 "	7	2	2
30 " 33 "	4	2	0
38 " 48 "	4	0	4
71 " 87 "	3	1	2

mation on this point was obtained. In most cases when a lamb reacted to $\alpha\beta$ toxoid both the α and β titres were increased, but, as the significance of the β complex is not clear, only the α titres were considered when studying the results.

Before the first injection of toxoid all the lambs up to the age of 33 days had titres of less than 1 unit; two of the lambs in the 38 to 48 day age group had titres of from 1 to 4 units and one had a titre of 17 units (this rose to 40 units following the injection of toxoid); one lamb in the 71 to 87 day group had a titre of 4 units.

All the lambs were bled and their sera were tested at from three to seven days after the last injection of toxoid.

The results of these experiments which are summarised in Table I. appeared to indicate that a response to toxoid could be obtained in some lambs at between 25 and 30 days old, but that some would not respond until they were about 40 days old.

As a longer interval between the injections of toxoid might give

a better immunological response the following experiment was carried out in 1946 on 30 lambs. Twenty were given injections of 2.5 c.c. toxoid when they were 25 to 27 days old and 5 c.c. toxoid when they were 34 to 36 days old. The remaining ten were left untreated as controls. Blood samples taken from all the lambs at 25 to 27 days, 34 to 36 days and 40 to 42 days old, were tested for their anti-haemolysin content with the results shown in Table II.

TABLE II
NUMBERS OF LAMBS WITH RAISED ANTI-HAEMOLYTIC TITRES

Group	Age in days	Numbers of lambs with titres of	
		1 to 9 units	Over 9 units
20 lambs receiving toxoid	25 to 27	2	0
	34 „ 36	1	1
	40 „ 42	1	2
10 untreated lambs ...	25 to 27	2	0
	34 „ 36	3	0
	40 „ 42	2	1

Thus, in this experiment, the injection of staphylococcal toxoid did not increase the number of lambs with raised anti-haemolytic titres when compared with the number of untreated lambs with increased titres. This result is at variance with the experiments conducted in the previous year. It is difficult to explain this difference, but the nutritional level of the lambs may have some influence on the formation of antibodies as the lambs in 1945 thrived better than those in 1946.

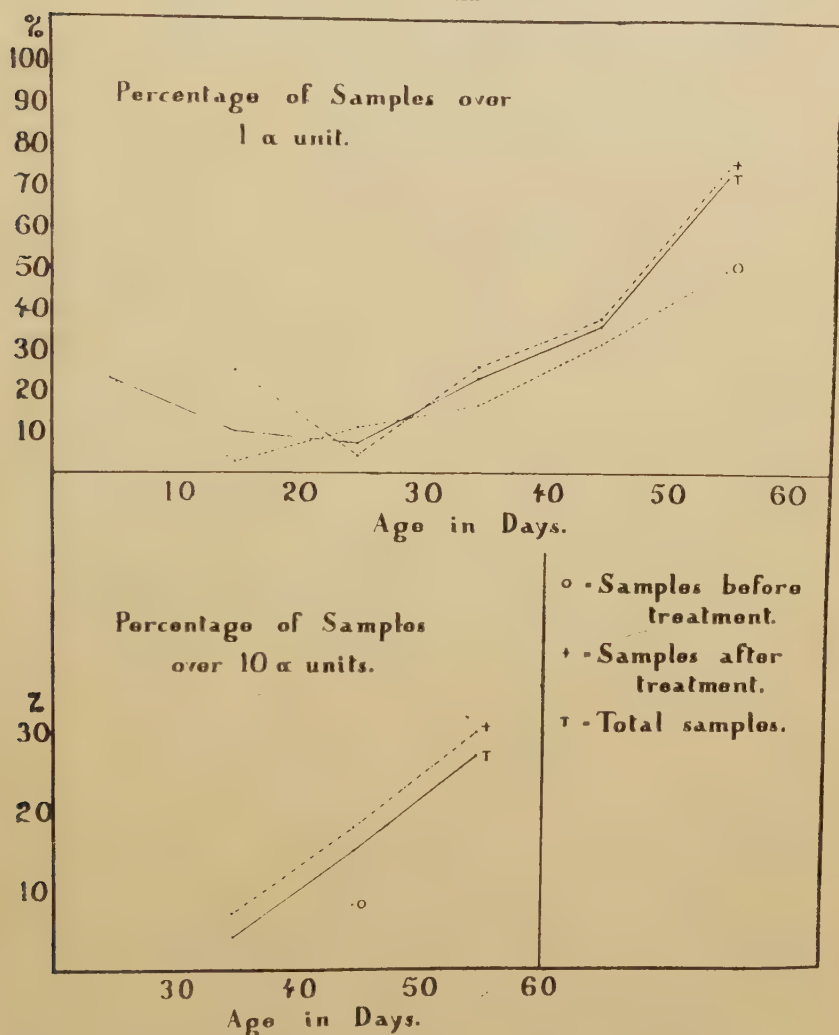
TABLE III

Age group	Number of samples with titres of 1 unit or under	Number of samples with titres of 1.1 to 10 units	Number of samples with titres of over 10 units
0 to 9 days	60	18	0
10 „ 19 „	38	4	0
20 „ 29 „	88	7	0
30 „ 39 „	62	16	3
40 „ 49 „	48	16	11
50 „ 59 „	6	10	6

Although the above experiments were inconclusive a study of the considerable number of blood-tests done during the course of the investigation indicates the general trend of staphylococcal anti-haemolysin production in young lambs. In all, 393 blood samples were tested: they were divided into groups according to the age of the lambs from which they were taken. The numbers with titres of 1 unit or under, 1.1 to 10 units and over 10 units respectively are given in Table III.

A number of these lambs received antigen either in the form of toxoid or as an intravenous injection of staphylococci. The percentage of the total number of samples which were over 1 unit, and also the percentage from untreated and from treated lambs over 1 unit were calculated for each age group. The variations are given as curves in Fig. 1 which shows that in the under ten days age

FIGS. 1 and 2



group 23 per cent. were over 1 unit. This percentage drops to seven in the 20 to 29 days age group and thereafter rises steadily. There is little deviation from this general trend in the samples from treated and untreated lambs. After the 30th day, the percentage over 1 unit from treated lambs tends to be higher and that from

untreated lambs lower than the percentage of the total. The significance for treated lambs at ten to 19 days, and for untreated lambs at 50 to 59 days, is questionable as these figures were calculated from small numbers.

The following experiment shows that the appreciable amount of antibodies in lambs under ten days old is due to passive immunity received from the colostrum. Four lambs were bled before they had sucked their mothers. The titres of all four sera were less than 0.1 of a unit. The lambs were again bled within seven days after they had sucked. The titres of the sera were now 0.7, 1.2, 1.4 and 1.0 units. The drop in the curve (Fig. 1) is presumably due to the elimination of these colostral antibodies. A possible explanation of the failure of young lambs to react to injections of staphylococcal toxoid may be that an active immunity cannot be established while traces of this passive immunity are present.

Fig. 2, in which the percentages of the total number of samples, and of those from treated lambs with titres over 10 units are plotted against the age groups, shows that the numbers with more than 10 units increase from the 30th day onwards. The percentage from treated lambs over 10 units is greater than that of the total number. Eight per cent. from untreated lambs were over 10 units between 40 and 49 days; this point is also indicated in the figure. Thus it would appear that the injection of antigen was responsible for the production of the majority of titres of over 10 units.

In order to test the resistance to staphylococcal infection of lambs which had received toxoid, a number of the immunised lambs were infected by intravenous injection of a staphylococcal suspension. Minimum infective doses for untreated lambs of 17 days old and about 40 days old respectively were determined by inoculating varying dilutions (1 c.c.) of strain 1468 into the jugular veins of groups of lambs of these ages. This dose was taken to be the dilution which produced symptoms of pyaemia within five days of inoculation, persisting for at least seven days.

The results of these determinations are given in Tables IV and V.

The minimum infective dose for lambs of about 17 days old was taken to be a 1 : 3,000, and for lambs of about 40 days old a 1 : 1,000, dilution.

Except for one lamb in the 17 day age group, which was given the 1 : 300 dilution and which developed pyaemia, the titres of all the lambs in these groups were below 1 unit at the time of infection.

Sufficient lambs were not available to fix minimum infective doses for the older groups of immunised lambs; however, four immunised lambs of from 57 to 66 days old and three untreated control lambs, aged 69, 70 and 71 days, with titres of less than 1 unit, were injected with 1 c.c. of the 1 : 300 dilution. Only one control lamb developed pyaemia. Thus the infective dose for this age group may have been too light.

On the other hand, when six lambs of from 75 to 95 days old, three of which were non-immunised controls with titres of less than 1 unit, were injected with 1 c.c. of a 1 : 20 dilution, the three controls developed pyaemia. In this case the infective dose may have been too heavy.

The results of infecting lambs which had been given injections of toxoid are shown in Fig. 3. In this figure the anti-haemolytic titre of each lamb at time of infection is plotted against the age of the lamb. The result of infection is indicated by the symbol on the figure. One lamb in the 75 to 95 day group showed slight lameness for two days and is indicated thus—"?" The titres of untreated control lambs which were infected are also included.

TABLE IV
MINIMUM INFECTIVE DOSE FOR LAMBS OF 17 DAYS OLD

<i>Dilution of No. 8 Brown's Scale suspension of Strain 1468</i>	<i>Number of lambs inoculated</i>	<i>Number of lambs developing pyaemia</i>
1 : 100	1	1
1 : 300	2	2
1 : 1,000	2	2
1 : 3,000	5	3
1 : 10,000	3	2
1 : 100,000	3	0

TABLE V
MINIMUM INFECTIVE DOSE FOR LAMBS OF 40 TO 42 DAYS OLD

<i>Dilution of No. 8 Brown's Scale suspension of Strain 1468</i>	<i>Number of lambs inoculated</i>	<i>Number of lambs developing pyaemia</i>
1 : 200	1	1
1 : 500	1	0
1 : 1,000	7	6
1 : 2,000	1	1
1 : 5,000	1	0

Fig. 3 shows that of 42 lambs with titres of less than 0.9 of a unit, 33 developed pyaemia and nine resisted infection; of 15 lambs with titres of 1 to 9 units, nine developed pyaemia and six resisted infection; and of 11 lambs with titres of over 9 units one developed pyaemia, nine resisted infection and one showed transient symptoms.

A more accurate result may be obtained by confining attention to the titres of the lambs which were infected when they were from 33 to 45 days old. All the lambs in this group received a 1 : 1,000 dilution of culture.

It would appear from Table VI that lambs with α anti-haemolytic titres of less than 0.9 of a unit are more susceptible to staphylococcal infection than those with more than 1 unit. That the immunity conferred by a raised titre is not absolute is shown by the fact

results of these experiments is the individual variations in susceptibility of lambs to infection by staphylococci. During the course of these experiments 45 lambs with titres of less than 1 unit were inoculated intravenously with the approximate minimum infective dose for their different age groups. Eleven of these lambs failed to develop pyaemia. In the group used to determine the minimum infective dose for the 33 to 45 day age group, one resisted infection with an inoculum which was twice the minimum infective dose for that group.

TABLE VI
INFECTION OF LAMBS AT 33 TO 45 DAYS OLD

<i>Titre in units</i>	<i>Number of lambs which developed pyaemia</i>			<i>Number of lambs which resisted infection</i>		
	<i>Immunised</i>	<i>Control</i>	<i>Total</i>	<i>Immunised</i>	<i>Control</i>	<i>Total</i>
<0.9	15	4	19	2	1	3
1 to 9	6	2	8	6	0	6
>9	1	0	1	3	1	4
Total	22	6	28	11	2	13

Similar variations in susceptibility of rabbits to staphylococcal infection are described by Julianelle (1944).

DISCUSSION

These experiments show that the production of anti-haemolysins in response to the injection of staphylococcal toxoid does not occur in lambs below the age of 25 days, whilst only a proportion of the lambs inoculated between 25 and 45 days old react. On testing by artificial infection, a smaller proportion of the reacting lambs than the non-reacting lambs developed pyaemia, but the presence of a raised titre did not necessarily indicate immunity.

TABLE VII

<i>Age in weeks</i>	...	1 to 2	2 to 3	3 to 4	4 to 5	5 to 6	6 to 7
Number of cases	...	1	11	16	16	9	1

Mean = 3.8 weeks. Standard deviation = ± 1 week.

During a survey of the incidence of tick pyaemia in the field estimates of the ages of 54 cases, when first reported, were made. The results are given in Table VII.

Thus the majority of naturally occurring cases are infected before they are capable of reacting to toxoid.

In the experiment already quoted (Foggie, 1943), an apparently significant degree of protection from tick pyaemia in the field was

obtained by the injection of lambs with staphylococcal toxoid. As there appears to be a discrepancy between the results of the experiments under discussion and the results of the 1943 experiment, a further examination of the conditions of the latter was made.

The management of the flocks on the Northern Ireland farms in the 1943 experiment differs from that most commonly practised on Scottish hill farms in that the ewes are lambed on low tick-free pastures and the lambs are not exposed to tick infestation until they are from two to four weeks old. During the experiment this period was extended until two injections of toxoid had been given, the lambs being then four to six weeks old : thus they received an injection and at lambing, were tested for the presence of anti-age they may have reacted and acquired immunity.

In order to facilitate identification in the 1943 experiment all ewe lambs were inoculated with toxoid, the ram lambs being left as untreated controls. In the course of the survey mentioned above the sex of 47 pyaemia cases was 30 males and 17 females (total 47) as compared with 2,104 and 2,184 respectively (total 4,288) amongst normal lambs on the same farms. The combined figures were : males 2,134, females 2,201 (total 4,335). This gives an $\chi^2 = 4.054$ and $P = 0.05$ to 0.02 .

Thus there appears to be a significant difference in sex susceptibility to tick pyaemia, ram lambs being more liable to be affected than ewe lambs. If this finding holds for Northern Ireland it reduces the validity of the results of the earlier experiment.

The method of infection may also have a bearing on the results of the two sets of experiments. A lamb with a moderately high titre may be able to withstand repeated small infections due to tick bite although it develops pyaemia when the infection is administered as a single inoculum.

EXPERIMENTS ON THE PROTECTION OF LAMBS AGAINST TICK PYAEMIA BY IMMUNISATION OF EWES WITH STAPHYLOCOCCAL TOXOID

An attempt was made to immunise ewes with staphylococcal toxoid to see whether sufficient passive immunity was given to their lambs to protect them against artificial infection with staphylococci.

METHOD

Eleven ewes were each given three injections of 2.5 c.c., 5 c.c. and 5 c.c. of toxoid at intervals of one week from February 15th to March 1st. A fourth injection of 10 c.c. toxoid was given as near to lambing as possible. The sera from blood samples taken at each injection of toxoid when they were over four weeks old. At this haemolysins.

One of the immunised ewes was not in lamb ; 13 lambs were born from the remaining ten ewes. One ewe had one blind teat and a poor yield of milk from the other ; her lamb died three days

after birth before a blood sample had been taken. Sera from the remaining 12 lambs were tested for anti-haemolysins during the first week of life and at intervals thereafter.

Seven of these lambs were given an infective dose of a 1 : 3,000 dilution of culture when they were from 12 to 18 days old.

RESULTS

The changes in anti-haemolytic titre in the ewes are shown graphically in Fig. 4. In general there was a good response to the injection of toxoid, although the first and second had more effect in raising the titres than the third and fourth. In most of the ewes the titre tended to drop rapidly after the peak was reached. The samples taken from these ewes at lambing showed that in eleven immunised ewes the mean titre was 12.09 units (S.D. $\pm$ 8.167 units) while in 38 untreated ewes it was 2.13 units (S.D. $\pm$ 2.482).

There is a highly significant difference between the means ($t = 6.360$, $P = > 0.001$). Thus the injection of staphylococcal toxoid increases the antibody content of ewes' sera. The mean of the anti-haemolytic titres of the colostrum of eight of the immunised ewes was 24.2 units ($\pm$ 13.93 units).

The anti-haemolytic titres of the lambs from these two groups of ewes are compared in the same way in Table VIII.

TABLE VIII
MEAN TITRES OF LAMBS FROM IMMUNISED AND UNTREATED EWES

Description of lambs		Number of lambs	Mean titre in units	Standard deviation in units
1 to 7 days old.	Ewes immunised	12	5.24	$\pm$ 5.459
1 " 7 "	" untreated	48	1.11	$\pm$ 1.393
12 " 18 "	" immunised	7	3.41	$\pm$ 2.066
12 " 18 "	" untreated	43	0.36	$\pm$ 0.518

Statistical analysis of these figures shows that there are highly significant differences between the two groups of lambs at both age levels.

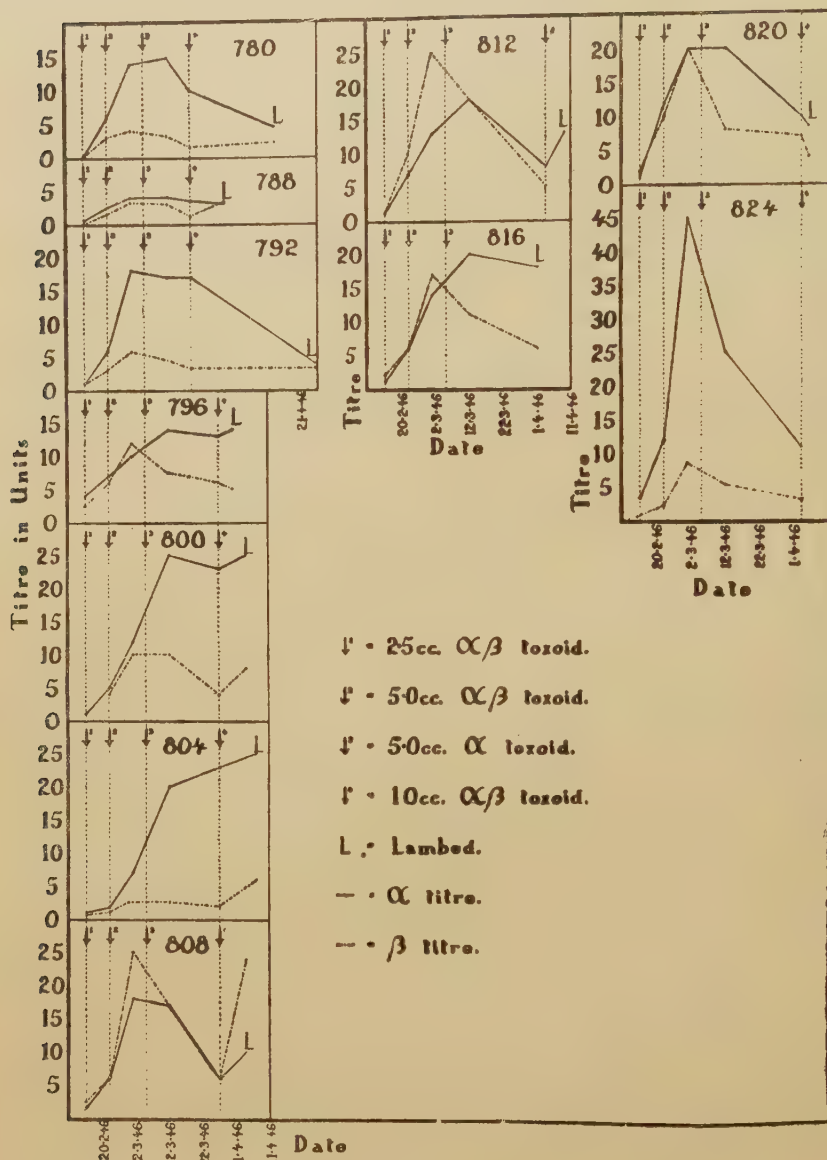
Seven of the lambs from the immunised ewes were given an infective dose of a 1 : 3,000 dilution of culture when they were 12 to 18 days old : five developed pyaemia.

As is shown in Fig. 5 there was a steady decline in anti-haemolytic titre from the first week of life onwards. Except in the case of lamb 919, in which symptoms of pyaemia persisted until 37th day of life, the infective dose did not appear to influence this decline. The two lambs which resisted the infection had titres of 7.0 and 1.7 α units respectively at the time of infection.

Although the results of this experiment gave little indication that the passive immunity conferred on the lambs from immunised mothers was sufficient to protect the lambs against artificially pro-

duced pyaemia, a field experiment was planned to test the effect of such an immunity against the naturally occurring disease.

FIG. 4

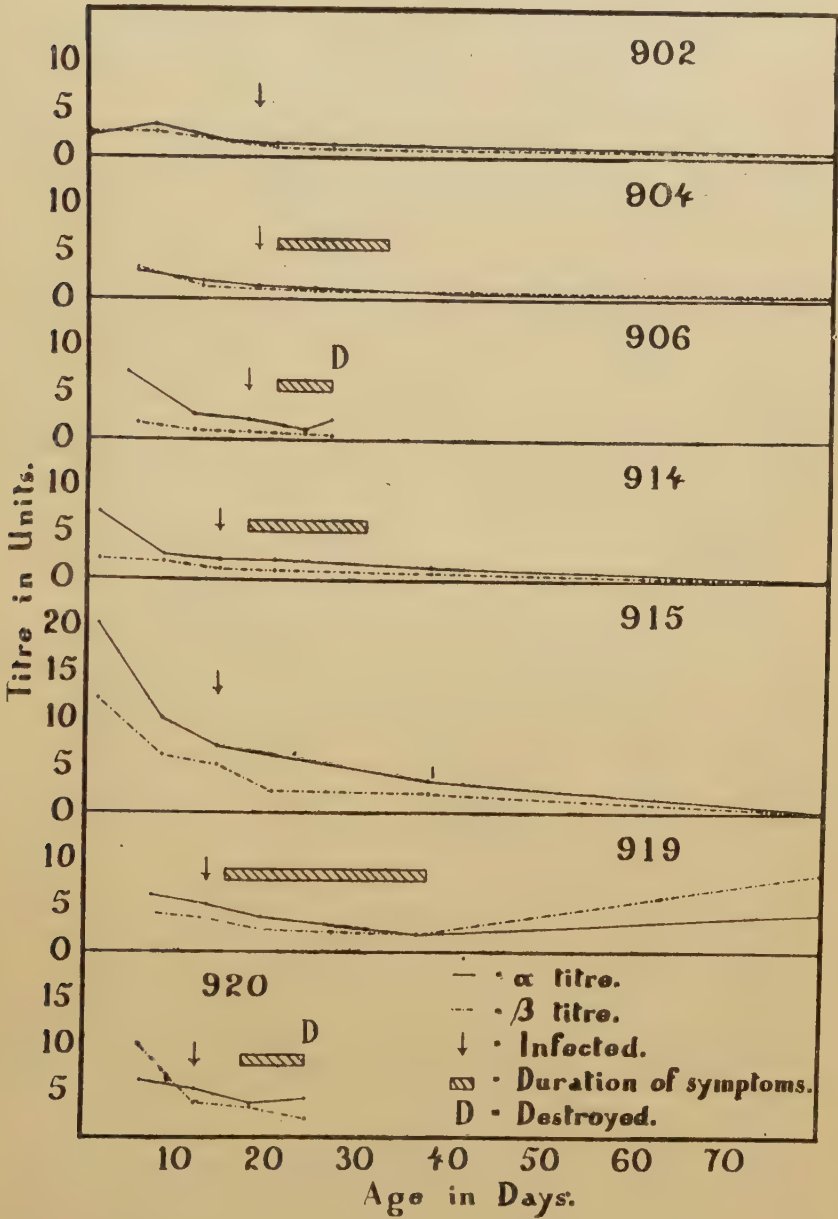


FIELD EXPERIMENT

A flock of 368 ewes on a Dumfriesshire hill farm was used. One hundred and eighty-two were given 'two injections of 5 c.c. α

staphylococcal toxoid prior to the start of lambing; the remaining 186 ewes were left untreated as controls. The first injections were

FIG. 5



made on April 1st and the second injections on April 10th. Lambing started on April 14th. As the two groups of ewes grazed on the

same pastures the immunised ewes were identified by marking them with keel.

Twenty immunised ewes were ear-tagged and blood samples were taken at each injection and on May 7th all but two of these ewes had lambed by this date. Blood samples were also taken from 20 ear-tagged ewes in the control group on April 1st and on May 7th, when they had all lambed. The sera were tested for their anti-haemolytic titres.

The mother of each lamb which developed tick pyaemia was identified and a note was made of the group to which she belonged. A final count of the lambs which had contracted tick pyaemia was made at ear-marking time on June 12th.

RESULTS

The results of the examinations of the blood samples from the two groups of ewes are summarised in Table IX which shows that the response to the first injection of toxoid was good (two of the immunised ewes had titres of 140 units at the second bleeding). Four weeks after the second injection, towards the end of the lambing period, the titres had fallen considerably, but they were still significantly higher than those of the control ewes. There was no significant change in the latter during the course of the experiment.

TABLE IX
MEAN TITRES OF ANTI-HAEMOLYSINS IN SERA FROM 20 IMMUNISED AND
20 CONTROL EWES

Date	Immunised ewes		Control ewes	
	Mean titre	Standard deviation	Mean titre	Standard deviation
1.4.46	3.4	± 2.82	1.8	± 1.59
10.4.46	51.0	± 35.74	—	—
7.5.46	13.8	± 7.29	2.1	± 2.54

The results of the counts of lambs which developed tick pyaemia in the two groups showed that there were six pyaemia cases and 175 normal lambs (total 181) from immunised ewes as compared with four pyaemia cases and 182 normal lambs (total 186) from control ewes. This gives $\chi^2 = 0.4692$ and $P = 0.5$.

In this experiment, therefore, there was no significant difference in the number of lambs which contracted tick pyaemia in the two groups.

DISCUSSION

The results of these experiments show that although ewes react to the injection of staphylococcal toxoid by a considerable increase in the anti-haemolytic content of their sera, sufficient immunity is not passed to their lambs to protect them against either artificial or natural infections of staphylococci.

There are two factors which may account for this failure.

1. The rapid drop in antibody content of the ewes' sera following the rise produced by the injection of toxoid. In order to obtain the maximum degree of passive immunity in the lamb the ewe would have to be given an injection of toxoid within eight or ten days of lambing. This is not practical under hill sheep farming conditions.

2. The rapid elimination of colostral antibodies from the lamb. As is shown in Fig. 5, the antibody content of the sera from most of the lambs from immune ewes drops to a low level within 15 days of birth. Tick pyaemia is a disease of lambs of from three to five weeks old and not of the newly born lamb. It may therefore be assumed that maternal immunity, which may protect the lamb early in life, has ceased to be effective when the lamb approaches the age of greatest incidence.

It is concluded from these experiments that staphylococcal toxoid, administered either to the lambs or to the ewes prior to lambing, is of no value in preventing the occurrence of tick pyaemia. A possible exception to this finding may be the injection of staphylococcal toxoid into the lambs on farms in which lambing is conducted on tick-free pastures, the lambs being later exposed to tick infestation. Further experiments are necessary to prove this point.

SUMMARY

Experiments are described which showed that lambs below the age of 25 days would not react to the injection of staphylococcal toxoid by the production of anti-haemolysins. Only a proportion of lambs between 25 and 45 days old reacted to toxoid.

It was found that lambs with raised anti-haemolytic titres were less likely to develop pyaemia when infected by intravenous injection of staphylococci than lambs with low titres.

The immunisation of ewes with staphylococcal toxoid prior to lambing was shown to be of no value in protecting their lambs against either artificial infection with staphylococci or naturally occurring tick pyaemia.

ACKNOWLEDGMENTS

I wish to thank Dr. J. Russell Greig, C.B.E, for his continued interest and encouragement.

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EXPERIMENTAL STUDY OF JOHNE'S DISEASE IN GOATS *

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INTRODUCTION

STUDIES on Johne's disease of cattle (*Enteritis chronica pseudo-tuberculosis bovis*) are hampered by the lack of a suitable experimental animal. Whilst bovines, being the most important natural host, are obviously indicated for the purpose, their use is perforce restricted by material considerations such as the cost, the time and the labour involved in following the necessary number of animals undergoing an essentially chronic condition. Attempts were made in the past to study the effects of *Mycobacterium johnei* in other species of ruminants known to be susceptible under natural conditions, such as sheep and goats. The disease can be set up in a certain percentage of these animals by parenteral administration of the specific organisms. The most likely route of infection under natural conditions is the oral route and yet there appears to be no data in the literature on the susceptibility of goats to Johne's bacilli administered per os. It seemed advisable, therefore, to carry out experiments in caprines in order to ascertain whether a progressive infection, similar to that occurring naturally in cattle, could be induced by that route. If this proved possible, it was hoped that certain aspects of the pathogenesis of Johne's disease, such as the portal of entry of the bacilli, their localisation during and after the period of incubation, their elimination from the body and the route by which the intestines become infected could then be investigated.

METHODS

Goats were collected from various sources and quartered on clean ground. Only those animals which failed to react to the tuberculin and johnin tests were used. The males were castrated.

Accommodation consisted of wired runs measuring 135 sq. yds., each surrounding a wooden shed, 4 sq. yds. in area, built on a concrete base. In summer the goats were tethered out singly or kept together in movable pens on cleared parkland (about 1 acre).

Diet was mainly composed of hay and a mixture of bran and oats. The amounts fed varied, but generally were not much in excess of maintenance requirements. Water was always available in pails.

Each experiment comprised a number of goats (6 to 12). They were fed and drenched with material containing Johne's bacilli. In the first three of the six experiments that were carried out intestinal scrapings from diseased cattle were used but these vary so much in their richness in bacilli

* The data reported in this paper were incorporated in a thesis for the Ph.D. degree of the University of London,

that it was felt advisable to ascertain whether cultures of *Mycobact. johnei* would be effective. In order to reduce to a minimum the effect of any variation of virulence that may exist in different strains of Johne's bacilli, two or four recently isolated strains (first to third generation) were used in the last three experiments. Cultures on Dunkin's medium were incubated at 38° C. for four to seven weeks and the growth weighed, ground finely in an agate mortar and administered in fractional doses per os as a drench and in the food (bran and oats) in the same way as in Experiments I, II, III. In the unweaned goats of Experiments V, VI, the bacilli were given in milk or a mixture of milk and gruel through a suckling bottle. Moreover, in Experiments IV and VI, one dose of inoculum suspended in about 1 ml. of sterile water was placed at the back of the tongue. In each experiment some animals were killed at intervals, while others died. A few unfed control goats were kept in contact with the treated animals to serve for comparison.

Observations were made during life on the development of allergy, the excretion of the bacilli in the faeces, the period of incubation and the symptoms presented. A detailed *post-mortem* examination was carried out as soon after death as possible. The tonsils, the submaxillary, pharyngeal, bronchial, mediastinal and the portal lymphatic glands, were collected into sterile Petri dishes. One portion of each was fixed in 10 per cent. formol-saline for histological study and the remainder served to make smears and cultures. The intestines were slit open throughout their length and laid flat on an enamelled table. Ten to 20 smears were prepared from various parts. Pieces were fixed and cultures made from scrapings of the washed mucous membrane. When necessary, the worm-burden was estimated by my colleagues, Dr. J. N. Oldham and Miss Myra L. Bingham.

The mesenteric lymphatic glands were denuded of fat or connective tissue and then cut in thin parallel slices with sterile razor blades; some portions were fixed and others used for smears and cultures. Guinea-pigs and cockerels were inoculated in some cases. After fixation, materials were embedded in paraffin and thin sections stained with haematoxylin-eosin and Ziehl-Neelsen methods.

Cultures were sometimes made directly from the organs but more often after grinding the tissue in presence of sand in sterile mortars, suspending in distilled water and treating volumes of 1.6 to 1.8 c.c. of the resulting suspensions with antiformin (available chlorine about 5.25 per cent.) to final concentration of 10 and 20 per cent. in sterile rubber stoppered centrifuge tubes. The antiformin was left to act at room temperature for 30 minutes, during which the mixtures were shaken at intervals and were afterwards diluted to about 12 c.c. with distilled water and centrifuged at 2,000 r.p.m. for 15 minutes. The supernatant fluid was discarded and the deposit in a few drops of saline or distilled water sown on to slopes of Dunkin's egg plus liver extract plus glycerinated extract of *Mycobac. phlei* (Minett, 1942). In addition to antiformin, other agents, such as 12 per cent. sulphuric acid, 5 per cent. oxalic acid and 4 per cent. sodium hydroxide were sometimes used.

In order to deal successfully with gram-positive sporulating contaminants, it was necessary to incorporate a selected violet dye up to a concentration of 1:10,000 in some tubes of the egg medium before inspissation as suggested by Dunkin (1928). One brand of Gentian violet out of eight

violet dyes tested was especially chosen for the purpose and has proved invaluable when dealing with intestinal scrapings or faeces.

The inoculated slopes were sealed with plasticine after 24 to 28 hours' incubation and maintained at 37° to 39° C. for at least three months, during which they were inspected regularly. Any suspicious colony was examined microscopically and further identified by its cultural requirements, *i.e.*, growth on the special medium and not on nutrient agar or Dorset egg medium.

EXPERIMENTS AND RESULTS

Six experiments have been carried out and are detailed in Table I.

Owing to limitations of supply and of available land and labour, only small numbers of goats could be used at a time. The experiments were started in December, 1941, and were completed by June, 1946, except for Experiment VI, one animal of which is still under observation. A few unfed control goats were kept with the treated animals to serve for comparison. In Experiment I, goats 7 and 8 were maintained on clean ground and far from any source of infection as out-of-contact controls.

The results obtained in the first two experiments showed that goats could be infected per os (Experiment I) and that resistance to infection by this route appeared to increase with age (Experiment II). Neither of the two adult goats of Experiment II (see column 8b) developed the disease in clinical form during the 14 months they remained under observation. Care was therefore taken in subsequent trials to use only young goats with a view to utilising their apparent greater susceptibility.

Among the treated goats observed for a reasonably long period (over nine months) the majority became clinical cases of Johne's disease. Reference to Table I, column 9, shows that out of 23 young goats in Experiments I, III, IV, V and VI, no fewer than 18 developed the typical symptoms and lesions. Among the five goats which had apparently proved resistant (2, 16, 28, 37 and 55), the *post-mortem* examination carried out on the first four showed that specific lesions and bacilli were present in the internal organs of two of them (16 and 28). It is therefore conceivable that had these animals been kept under observation for a longer period, some might have succumbed to the infection. The fifth animal, which is apparently resistant, is still alive and in good condition.

The weight of bacilli needed to infect the great majority of young goats was found to be relatively small amounting to 24 to 43 mg. of moist growth given in the food or as a drench in three to eight doses spread over one to two weeks. The quantity required was easily grown on slopes of the special egg-medium from strains of very recent isolation.

Of the unfed control goats one (G1) remained in good condition throughout the 16 months' observation, and the two others (30 and 31) were still alive more than three years after the beginning of the

TABLE I
SUMMARY OF EXPERIMENTS

1	2	3	4	5	6	7	8	9	10
Experiment No.	Date	No. of Goats used	Age in Months	No. Artificially Infected	Infective Material	No. of doses given	Fate of Artificially Infected Goats		Fate of Controls
							(a)	(b)	
I	17.12.41	Eight Nos. 1-8	6	5	Intestine scrapings	3	1	3	The three remained healthy. Nos. 7 & 8 used in Exp. II
II	13.11.42	Six Nos. 7, 8, 9, 10, 12 and 13	12-18	4	Intestine scrapings	3	2	0	The two remained well. No. 8 used in Exp. III
III	21.6.43	Six Nos. 14, 15, 16, 18, 19 and 20	6	6	Intestine scrapings	4	4*	1	No controls used
IV	9.11.43	Eight Nos. 21-28	4-5	7	Culture 45 mg.	4	4	2	One control died early of coccidiosis
V	29.4.44	Ten Nos. 29-38	1	8	24 mg.	8	2	5	Two controls, one dying of J.D.
VI	19.4.45	Twelve Nos. 50-61	1	12	43 mg.	3	4	7	No controls used
								8	

Column 7: The infective material was given over a period of a week except in Exp. V where the administration lasted a fortnight.

Column 8: (a) Shows the number of goats artificially infected which had died or had been killed within six months of the beginning of the experiment with a view to studying the distribution of the bacilli intra-vitam.

Column 9: (b) The numerator represents the number of goats which had become clinical cases of John's disease out of the total (denominator) of animals observed for nine months or over.

* or 5 if Goat No. 8 is included.

experiment. One goat (30), however, has been steadily losing weight, reacting to the johnin test and eliminating Johne's bacilli in its dung. When killed on the 17.6.47 it showed extensive lesions of Johne's disease.

As similar experiments on calves were not carried out simultaneously, the susceptibility of these animals compared with that of goats can only be assessed indirectly by reference to feeding experiments reported in the literature. With huge doses of natural material, several workers (Bang, 1906; Malm, 1911; Miessner and Trapp, 1910; Twort and Ingram, 1913; M'Fadyean and Sheather, 1916; McIntosh *et al.*, 1934; Hagan, 1938; McEwen, 1939, etc.) have succeeded in reproducing the disease in a percentage of calves. These authors emphasise the relative difficulty in regularly inducing the disease in a clinical form in cattle.

There appear to be no records of normal calves being fed with graded doses of Johne's bacilli and, in general, the drastic methods used in most of the cases are in marked contrast to the relatively milder ones in these experiments on goats. The advantage of using cultures as infective material instead of intestinal scrapings is that more uniformity in dosage can thus be attained and this is bound, in some cases, to be reflected on the results.

The experiments reported herein were few and comprised a relatively small number of animals, yet the results (summarised in Table I), appear sufficiently definite to suggest that goats, preferably very young ones, are readily susceptible to the experimental disease. The latter, moreover, bears such a close resemblance to that observed in bovines naturally or artificially infected that goats might quite well be used experimentally to study various aspects of Johne's disease still requiring elucidation.

No dogmatic conclusions can be drawn from the experiments as regards the effect of age on susceptibility. Some evidence that adult goats are more resistant than young ones to infection may be adduced from the results of Experiment II.

SYMPTOMS AND COURSE OF THE EXPERIMENTAL DISEASE

The symptoms of the experimental disease are similar to those recorded in natural outbreaks in goats and cattle. After a period of incubation which lasted six months or longer, the first symptoms noted were unthriftiness and progressive loss of weight. The normal "pellet-form" appearance of the faeces was altered; intermittently they became "sausage-like" in shape and soft or mashy in consistency. Fluid diarrhoea, so commonly observed in cattle, was rather exceptional and limited to the very last stages. The relatively dry diet on which the animals were maintained may account for this, for when a grass supplement was available, there was a greater tendency to scour, though even then the diarrhoea was rarely continuous. In many cases the faeces were not obviously abnormal in form,

A peculiar foetid, sickly and rather objectionable smell, very distinct from the normal aromatic odour of the dung, was present in clinically affected goats. Faeces were also at times coated or mixed with streaks of mucous and in two instances entire mucus plugs very rich in Johne's bacilli and resembling portions of foetal membrane were excreted per rectum.

The general appearance of the animal changed as the disease advanced. The coat became untidy and somewhat dull or discoloured. General wasting and emaciation, affecting more particularly groups of muscles of the hind quarters, shoulders and cervical regions, were visible. Signs of progressive anaemia were generally present and oedema in the "jowl" with deformation and puffiness of the intermaxillary space were seen in isolated cases. The head, especially in the advanced stages, appeared at times larger than normal with a rugged profile, but the eyes remained bright and alert as a rule. More water was drunk by the affected animals. The appetite was good throughout except in the last stages when it became capricious. Aggressiveness towards other goats was often shown more especially at meal times.

The duration of the experimental disease ranged from about 10 to 24 months or longer. In many cases the animals were killed before the terminal stages. As was stated earlier, some goats which had been artificially infected did not show appreciable clinical symptoms during the period they remained under observation (see Experiment II, Table I). The case of goats 2, 16, 28, 37 and 55 in other experiments is of interest; for a time they showed by their allergic response and excretion of Johne's bacilli in the faeces and temporary loss of weight, that they had in fact become infected, but later on they appeared to shake off the infection. Complete "bacteriological" recovery, however, cannot be said to have occurred in *all* these cases, as in two of them (6 and 28) specific lesions and living bacilli were found at *post-mortem* examination.

Goats in the clinical stages of the disease appeared to show less resistance than the controls to parasitic infections, such as coccidiosis and strongylosis. When the latter threatened the life of the animal, phenothiazine treatment and hygienic measures (frequent changes of litter and sometimes fresh grazing) had to be instituted.

ALLERGY IN EXPERIMENTAL GOATS

Technique

Animals under experiment were periodically tested with two johnin products.* One was a P.P.D. preparation (labelled J.28), preserved in powder form, and made up as required to a concentration of 3 mg. per ml. in borate buffer of pH 7.3. The other product (J.33) was a stable, heat-concentration synthetic medium

* Kindly supplied by R. E. Glover of the Medical Research Council Farm Laboratories, Mill Hill, N.W.7.

Legend : A = Allergic tests : two Johnins used simultaneously (see text). Reactions to Johnin P.P.D. are given first, followed by those of the heat concentrated Johnin. P = positive ; S = suspicious ; N = negative.
 B = Cultural examination of faeces carried out at about the same dates as the Allergic tests ; + = Johnne's bacilli isolated ; — = Johnne's bacilli not recovered ; . = cultures not carried out ; C = contaminated cultures.
 * Goats 7 and 8 acted as out-of-contact controls in Experiment I. Goat 7 was artificially infected with goats of Experiment II on 13.11.42—Goat 8 acted as in-contact control in Experiment II and was artificially infected on 21.6.43 with other goats of Experiment III.

TABLE III
EXPERIMENT II (Started 13.11.42)

Date of Examination	1942				1943						1944			
	2.11	12.12	16.1	13.2	15.3	29.4	17.5	3.8	1.9	27.9	27.10	27.11	5.1	14.2
Goat No.	A	SN	NN	S?P?	NS	NS	NN	NN	PP	PP	PP	PP	PP	
	B	.	C	C	C	.	C	—	—	—	—	—	—	
9	Killed 18.1.44 Good condition P.M. : + Avian tubercle bacilli + Johne's bacilli													
10	A	NN	NN	NN	NS	NN	SS	Killed 5.5.43 Good condition P.M. : Negative						
	B	.	C	C	C	.	C							
12 Control	A	SN	NN	NS	NN	NN	SN	NN	Killed 8.7.43 Good condition P.M. : Negative					
	B	.	C	C	C	.	C	.						
13	A	NN	NN	NN	NN	NN	NN	SS	NN	NN	NN	NN	NN	NN
	B	.	C	C	C	.	C	—	—	—	—	—	—	—
Killed 8.3.44 Good condition P.M. : + Johne's bacilli														

For Legend see Table II—Contaminated media were largely responsible for the bad results in the first four cultural tests.

johnin which had been filtered and preserved with merthiolate (1:10,000 final concentration). The keeping qualities of the P.P.D. in solution and of J.33 were quite good but, as a rule, solutions of the former were not kept longer than three months.

Tests were carried out simultaneously with the two johnins at intervals of four weeks or more starting one month after artificial infection until death or slaughter. From time to time avian and mammalian tuberculin tests were also made for purposes of comparison. The technique was that of the double intradermal method, the two johnins being injected in 0.1 ml. doses on opposite sides of the neck. A diffuse oedematous swelling with an increase to at least twice the original thickness of the skinfold was interpreted as a positive reaction, more emphasis being placed however on the character of the swelling than on the actual measurement. An appreciable increase in thickness to less than twice the original size with a rather circumscribed swelling was considered doubtful or suspicious. Otherwise, the reaction was termed negative.

Results

The results are tabulated under each experiment (see Tables II to VII inclusive). It can be seen that the pre-allergic period varied somewhat and, as a general rule, lasted one to six months. In Experiments II and III (Tables III and IV) the allergic response was somewhat irregular in its onset and course, but proved to be much more uniform in the later experiments when the goats were infected with cultures of *Mycobact. johnei* instead of intestinal scrapings.

Most of the goats which became reactors remained allergic for a long time. There were isolated cases, however, where reactions to johnin grew less intense and disappeared altogether for a time and then reappeared at irregular intervals. The various patterns of allergic response may be depicted as follows :—

Pattern I	NSPPPPPPS
II	NNSPPSNNN
III	NNSNSNNNN
IV	NNNSNPPPP
V	NNNNNP NPPNPP.....

where each letter denotes the result of a johnin test (P = positive: S = doubtful: N = negative:) at intervals after infection. Animals which in the long run developed the clinical disease reacted according to Pattern I, and more rarely to Pattern IV which really represents a variant with a prolonged pre-allergic period (see Goat 20, Table IV). Goats which shook off their infection generally followed Pattern II (see Goats 2, 16, 37 and 55). Resistant animals were represented by Pattern III and reactions of the only three in-contact control goats have either followed Pattern II (see Goat 1) or Pattern V (see Goats 30 and 31.)

TABLE IV
EXPERIMENT III (Started 21.6.43)

Date of Examination	1943										1944									
	17.5	15.6	3.8	1.9	27.9	27.10	27.11	8.12	5.1	14.2	18.3	19.4	17.5	21.6	29.7	25.9	28.10	2.12		
Goat No.																				
14	A	NN	.	SN	NN	NS	NN	NN	N?	Died 9.12.43 of strongyle infection										
	B	.	.	-	+	-	+	+		P.M. + + Johne's bacilli recovered										
15	A	NN	.	Died 13.7.43																
	B	.	.	Coccidiosis and strongyle infection																
16	A	NP	.S	SS	NN	NN	PP	P?N	S.	NN	NN	NS	NN	.N	NN	NN	NS	NS	Killed 5.12.44	
	B	.	.	-	+	+	+	+	.	-	-	-	.	-	-	-	.	-	Good condition P.M. : ++	
18	A	NN	.	Died 26.7.43																
	B	.	.	Coccidiosis																
19	A	NN	.	Died 22.7.43																
	B	.	.	Coccidiosis																
20	A	.	NN	SN	SN	NS	SS	NN	N.	NN	PP	PP	PP	.P	PP	PP	PP	PP	Killed 8.11.44	
	B	.	.	-	-	-	+	+	.	-	+	+	+	.	+	+	+	+	Condition fair, losing weight. Clinical case P.M. : +++	

For Legend see Table II.

Tables II to VII show that the reactions to the two johnins were on the whole comparable. The heat-concentrated product proved to be slightly less specific than the P.P.D. but not to an extent that rendered interpretation difficult.

Various preparations of avian tuberculin were compared with the two johnins at intervals. Though the number of such tests was not large, the results obtained strongly suggested that under the experimental conditions prevailing the johnin preparations were superior in specificity and potency to the avian tuberculins. Furthermore, mammalian tuberculin rarely elicited a specific response from goats affected with Johne's disease.

The "lag-phase" in the development of allergy in the experimental goats varied between about one month, the shortest period after infection at which tests were made, and six months, a result which is comparable with the findings of others in cattle. Thus, Miessner and Trapp (1910) observed a positive reaction to avian tuberculin in two calves which had been fed infected intestinal scrapings five to six months previously, and Malm (1911) recorded a nine-week lag in a 14-day-old calf similarly fed. Houthuis (1932) and McIntosh *et al.* (1934), who respectively tested one and three calves with johnin by the double intradermal method, noted pre-allergic periods lasting six and four months. Similarly, Hagan (1937, 1938) stated that bovines which had been infected per os react sharply within four to six months to johnin or avian tuberculin injected intravenously. All these workers used infected intestines in their experiments. The results recorded in this paper which relate to goats infected with intestinal scrapings or with cultures confirm the slowness with which allergy develops in this disease. Many factors may influence its onset, among which dosage and age would seem to be important (see Experiment II.)

In the writer's opinion, the doubtful or suspicious reactions which were sometimes noted in the goats before the onset of clear cut positive reactions represent in many cases a stage in the development of allergy. As stated above, the technique of the test and the johnins used remained the same throughout the experiment. The differences in readings which were observed on the same animal at different periods after infection suggest that the process of sensitisation is a gradual one. It would have been interesting to follow the fluctuations of allergy more closely by studying the response to various dilutions of johnin but it was not considered desirable to introduce another variable factor into the experiments.

It is well known that in some clinical cases of Johne's disease in cattle, johnin reactions are very slight or negative. In contrast to this, it can be seen from Tables II to VII that all the experimentally infected goats which developed symptoms of the progressive disease remained allergic for a long time, and it was only in the very advanced stages that the response to johnin tended to diminish.

TABLE V
EXPERIMENT IV (Started 9.11.43)

Date of Examination		1943					1944						
		22.9	8.12	5.1	14.2	18.3	19.4	17.5	21.6	29.7	25.9	28.10	
Goat No.													
21	A	NN	Died 3.12.43										
	B	.	Coccidiosis										
22	A	NN	PP	SS	SS	PP	PP	.P	Killed 20.5.44 Condition fair P.M. : +				
	B	.	—	—	—	—	.	—					
23	A	NN	NN	SP	NS	SN	PP	.S	SP	.P	PP	PP	
	B	.	—	—	+	+	.	—	.	+	+	+	
24	A	NN	PP	Died 11.12.43 Coccidiosis and strongyle infection P.M. : Negative									Killed 14.11.44 Condition very poor Clinical case of J.D. P.M. : +++
	B	.	—										
25	A	NN	PP	SS	PP	PP	Killed 24.3.44 Condition good P.M. : ++						
	B	.	—	—	+	+							
26	A	NN	PP	P?	PP	PP	PP	.P	PP	PP	PP	Killed, dying, 24.10.44 Condition very poor Clinical case P.M. : +++	
	B	.	—	+	+	+	+	—	.	+	+		
27	A	NN	PP	Died 11.12.43 Coccidiosis									
	B	.	C										
28	A	NN	PP	PS	NN	NN	NN	.N	PP	N.	PP	PP	
	B	.	—	—	—	—	.	—	.	+	+	C	
												Killed 31.10.44 Condition very good P.M. : ++	

For Legend see Table II.

TABLE VI. EXPERIMENT V (Started 29.4.44)

Date of Examination		1944										1945										1946										1947																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
		4.4	21.6	29.7	25.9	28.10	2.12	2.1	14.2	16.5	23.6	15.8	3.10	27.11	2.2	4.5	20.8	12.10	20.1	29.3																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								

TABLE VII
EXPERIMENT VI (Started 19.4.45)

Date of Examination	1945					1946				1947		
	16.5	23.6	15.8	3.10	27.11	2.2	4.5	20.8	12.10	20.1	29.3	
Goat No.												
50	A	NS	SS	PP	.P	.P	PP	PP	PP	PP	P	Killed 17.4.47 Clinical case P.M. : +++
51	A	NS	NS	PP	P.	.P	PP	PP	PP	Killed 23.10.46.	Thin. P.M. : —	Strongyles and John's disease
52	A	Killed 15.5.45. Condition good. P.M. : +										
53	A	NN	NN	Died 11.7.45. P.M. : +								
54	A	NN	NN	PP	P.	P.	PP	PP	Killed 4.6.46. Clinical case.	Very emaciated P.M. : +++		
55	A	NS	NN	NN	.N	PP	PP	NS	PP	SN	SP	N. Still alive Condition good
56	A	NN	NN	PP	.N	PP	PP	PP	Killed 2.8.46. P.M. : +	Condition very poor.		
57	A	NS	PP	Killed 5.7.45. P.M. : +								
58	A	SS	NN	NN	S.	.P	PP	PP	PP	Killed 24.8.46. Clinical case.	Very thin. P.M. : +++	
59	A	NS	NS?	PP	.P	.P	PP	PP	P.	Killed 16.11.46. Clinical case.	Very thin. P.M. : +++	
60	A	SS	NN	PP	.P	P.	SS	NN	PP	PP	P.	Killed 10.4.47 Very thin P.M. : +++
61	A	Died 7.5.45. P.M. : —										

For Legend see Table II. In this experiment cultural tests on faeces were not carried out as a routine.

Successive tests were sometimes, though seldom, contradictory in the sense that a negative or suspicious reaction was recorded between two positive ones. In other words, some reactors ceased to react for a time, *e.g.*, in-contact controls (30 and 31) and infected goats (23, 25, 28, 56). Bang (1914), Hagan (1938), Ware and Srinivasan (1942) reported similar responses in cattle to avian tuberculin and M'Fadyean *et al.* (1916), Minett (1935), and Hagan (1938), to johnin by various routes. The difficulty of interpreting with confidence a single negative allergic test is thus shown to apply to goats as it does to cattle. Further, even when a series of negative reactions is obtained, it is not justifiable at present to assume that the animal is not harbouring the specific bacillus (see *post-mortem* findings on 13, 14 and 16), or shedding it in the faeces (see 14 and other goats in the first few months following infection). Positive johnin reactions in the experimental goats were more significant. Thus from a reactor, Johne's bacilli could as a rule be isolated from the faeces during life or the internal organs at *post-mortem*. False positive reactions associated with micro-organisms other than *Mycobact. johnei* were not encountered here and did not therefore create confusion such as occurs when testing cattle. It is true that a strain of the avian tubercle bacillus was isolated from the mesenteric lymph glands of Goat 9, a johnin reactor, but whether sensitisation was attributable to this organism or to Johne's bacillus which was recovered from the animal's tonsils and pharyngeal lymph glands is a matter for speculation.

The allergic response of young goats to johnin was such as to suggest that when sensitised cattle are not available, they might be suitable for the testing of johnin preparations prior to their use in the field.

THE ELIMINATION OF JOHNE'S BACILLI IN THE FAECES

Judging from data available in the literature, this question has not yet been thoroughly investigated. Malm (1911) failed to demonstrate excretion of the organism by two calves for periods up to 12 and 21½ months respectively after experimental feeding, although both animals were then showing clinical symptoms of the disease and the second one had been doing so from 15 months onwards. McIntosh *et al.* (1934) infected three young calves and reported the presence of "bacilli resembling Johne's bacilli one month after infection followed by negative findings, for respective periods of 4½, 7½ and 13½ months, since when such bacilli have been constantly present while they have been detected in only one smear of a control." McEwen (1939), using large quantities of material containing the ovine strain of *Mycobact. johnei* in repeated doses given to six calves during a period of ten months, recorded the presence of acid-fast bacilli not always arranged characteristically in clumps, for a few months before the onset of symptoms and intermittently during the clinical stage of fatal cases. Many years previously,

Meyer (1913) claimed that in the clinical stages the excretion is intermittent.

All the above findings were based on the examination of smears, a technique which is open to criticism and which is known not to be very reliable.

It was thought advisable therefore to try cultural methods on dung samples from goats at various intervals after infection in the hope of collecting the necessary data.

TECHNIQUE

Two to four pellets of dung or their equivalent were obtained per rectum, ground in a sterile mortar and suspended in about 15 ml. of sterile distilled water. The suspension was allowed to settle for 10 minutes on the bench and a measured volume (1.6 to 1.8 ml.) of the supernatant fluid was transferred to sterile stoppered centrifuge tubes and treated with antiformin in the manner described in the chapter on methods. If this did not suffice to eliminate contaminants, the operation was repeated using a thinner suspension of faeces or a stronger concentration of antiformin.

RESULTS

Tables II to VII inclusive show the results of the periodical cultures and correlate them with the allergic response. It must be stated that in Table III (Experiment II) the first five cultural tests were vitiated by batches of contaminated media due to a sporulated bacillus which had withstood the process of sterilisation. Further trouble through this cause was prevented by autoclaving the mixture of Dunkin's liver extract and glycerinated *Mycobact. phlei* for 15 minutes up to 120° instead of 115° C.

Not included in Table II is the fact that Johne's bacilli were isolated from all the five infected goats of Experiment I four days after the last infective meal. Eleven days after the latter, bacilli were obtained from one animal only out of the five examined. In each of Experiments IV and VI also similar attempts were made on three goats after they had been infected with culture of *Mycobact. johnei*, but in no case was the causal organism isolated. It is likely that most of the bacilli are destroyed during the passage through the digestive tract and only when large doses are given as in Experiment I can they be detected in the faeces soon after infection by the technique employed, and then only for a short period. This preliminary stage appears to be followed by a "silent" period lasting a few weeks or months, after which they re-appear in the faeces in large enough numbers to be detected again. These bacilli are evidence of active invasion of, and multiplication in, the intestinal mucous membrane. The objection that the experimental goats, having been kept on infected ground, were likely to be constantly picking up Johne's bacilli and that some of the latter could have made their appearance in the faeces must however be borne in mind. But this possibility was found to be of theoretical importance only

and can be ruled out because of repeated failures to isolate Johne's bacilli with the same technique from the faeces of healthy in-contact control goats. In every case in which positive results were obtained from the latter they could be correlated with evidence of active infection as judged by positive johnin reactions. It seems reasonable therefore to interpret a positive cultural finding as generally implying that the bacilli were at the time of the test being excreted from a lesion or lesions. The latter, judging from the clinical and pathological findings, may extend or retrogress and accordingly are followed by the typical disease or by apparent or even true recovery (see *post-mortem* findings on 1, 2, 16 and 37).

Negative cultural results on faeces do not, in contrast, necessarily indicate that bacilli are not being eliminated. Repeated failures to isolate the organisms, however, have generally proved to be significant in suggesting that the disease was temporarily checked or overcome.

In goats showing symptoms of Johne's disease, excretion of the bacilli, judging by Tables II to VII, appears to be a steady process of variable intensity, as shown by the numbers of bacilli recovered, but continuous in character. No evidence of its intermittence in the progressive fatal disease has been obtained.

As was stated earlier, during the incubation period and long before the onset of symptoms, bacilli could be found in the faeces, but the extent, course and duration of this bacillary excretion depends on the susceptibility of the animal, *i.e.*, on the play of a varied set of factors which control whether an animal is going to develop the clinical disease, recover completely or maintain a host-parasite relationship on more or less advantageous terms.

There appears to exist in the experimental goat a close correlation between the results of the johnin test and the presence of bacilli in the dung. For diagnostic purposes the balance on the whole is in favour of the allergic test. Considering the relative crudeness of the antiformin method of culture used, it is surprising that a correlation was achieved as close as is suggested by Tables II to VI and that in some instances bacilli were recovered before the johnin reaction had become positive.

The literature contains little information on the excretion of Johne's bacilli in apparently healthy cattle under natural conditions. In order to find out whether the results obtained in experimental goats apply also to bovines, faeces from 24 beasts in three attested herds (where Johne's disease had been observed) were collected and cultured after treatments with antiformin. Three animals which had not shown previously any specific symptom yielded positive cultures of *Mycobact. johnei*. Two of these were reactors to the johnin and/or avian tuberculin tests. The third animal, a young heifer, was a non-reactor at the time of the cultural test but has since become allergic. Whether it will become a clinical case or not is a question that cannot be answered at present, but it is still under observation.

LESIONS IN THE EXPERIMENTAL DISEASE

Nature of the lesions

The nature of the changes seen in goats experimentally fed with Johne's bacilli did not differ from those already reported in goats by various workers (M'Fadyean and Sheather, 1916; Holmboe and Slagsvold, 1934; Eveleth *et al.*, 1943; Pande, 1942) or in cattle. They will not be described here in detail.

The changes in the intestines (see Plate I, Figs. 1 and 2) were similar in distribution and extent to those of cattle. They were, however, often more difficult to detect with the naked eye and in many of the early cases would have been missed if histological sections or cultures had not been carried out.

The mesenteric lymphatic nodes in the clinically affected goats were usually oedematous: they exuded a greyish-brown and sometimes milky fluid from a fresh section. In 10 animals out of 18 they were definitely enlarged. Serial slicing revealed no abnormalities in 9 goats, but in the remainder the nodes contained degenerative foci in the form of caseous or calcified nodules measuring about 0.5 to 5 mm. in diameter, arranged mainly at the periphery (see Plate I, Figs. 3 and 4). The surface of the gland was sometimes bosselated through their presence. The capsule was sometimes markedly thickened and afferent lymphatic vessels could be easily seen. Elsewhere than in the intestines and mesenteric nodes, macroscopical changes were absent except for those associated with emaciation and anaemia.

Histologically, the intestinal lesions were similar to those in cattle. In the mesenteric nodes also there were islets of epithelioid and giant cells. These cells in the glands showed a tendency to localise in the lymphatic sinuses where they progressively formed syncytial masses. The whole structure of the gland in advanced cases became barely recognisable because of the disappearance of the normal follicles. Invasion of the capsule and the walls of lymphatic vessels with epithelioid cells, lymphocytes and fibroblasts was not rare. In some of the goats, some epithelioid cells present in the nodules showed well marked degeneration in the form of caseation and calcification, as illustrated in Plate I, Figs. 3 and 4. Usually large numbers of Johne's bacilli were present in smears or sections of these nodules.

The tonsils, the submaxillary, pharyngeal and portal lymph glands also showed specific histological changes in a variable percentage of clinical cases (see Table VIII, and Plate II, Figs. 7 and 8), but caseation or calcification were not encountered in these organs.

The peculiar mesenteric lesions detailed above—peculiar in so far as similar changes are not found in this disease in cattle—had been previously observed by M'Fadyean and Sheather (1916). Holmboe and Slagsvold (1934), Pande (1942) in goats, and by Jarmai (1922) in a gnu (*Connochaetes albojularis*, Thom). Evidence that *Mycobact. johnei* had been involved was obtained by M'Fadyean and

Sheather and by Jarmai, and the former authors also succeeded in reproducing the mesenteric lesions experimentally by parenteral injection of cultures of Johne's bacilli. These cultures proved however to be slightly contaminated with tubercle bacilli and the results, although suggestive, needed confirmation. The disease of goats which Holmboe and Slagsvold investigated was somewhat atypical as all their attempts to grow the causal organism failed, but they reported that the inoculation of material into small experimental animals was negative.

No difficulty was experienced in isolating Johne's bacilli from the mesenteric lymph glands of 15 clinical cases of Johne's disease experimentally induced in goats on which cultural tests were made. The presence of *Mycobact. tuberculosis* in the caseous and calcified nodules was ruled out by the failure to isolate tubercle bacilli on culture media such as plain and glycerinated Dorset Egg slopes, and in addition, animal inoculations gave the following results :

Mesenteric glands of: Animals inoculated: Post-mortem findings:

G20	2 guinea-pigs, subcutaneously.	Killed after 5½ and 7 months — negative.
G32	„	„ „
G35	„	Killed six weeks and four months after inoculation — nega- tive.
G36	2 guinea-pigs, subcutaneously, and 2 chickens, I.M.	Killed after 4 and 5½ months — negative.

The degenerative changes found in the mesenteric nodes of goats suffering from Johne's disease could be confused with tuberculous lesions, but a differential diagnosis could be made by a study of the distribution of the lesions and their histology, and by the isolation and identification of the organism present. For interest's sake, Plate II, Fig. 6 is appended showing a section of mesenteric gland of Goat 9 from which the avian type of *Mycobact. tuberculosis* was isolated. This animal was not clinically affected when autopsied. Lesions appeared to be limited to one mesenteric gland which was enlarged and contained two small calcified nodules. From these, as well as from the apparently normal mesenteric glands, *Mycobact. tuberculosis avium* only was recovered in large numbers. Johne's bacillus was not isolated from the above-mentioned glands or from the intestinal mucous membrane, but proved to be present in the tonsils and pharyngeal nodes.

In some goats caseous or calcified foci shown macro- and microscopically to be of parasitic origin were encountered in the mesenteric nodes. They were single or multiple, usually circular in shape, well encapsulated and easy to enucleate. The identity of the parasite concerned has not been determined.

Distribution of lesions and bacilli in experimentally infected goats

It is usual to think of Johne's disease as a localised affection, the causal organism invading the intestine and its associated lymphatic glands and showing no tendency to generalisation. In M'Fadyean's words (1918) "the bacillary invasion appears usually to begin and end in the intestines and the group of lymphatic glands in immediate connection with them." This opinion, to which most early workers subscribed, was based on the failure to demonstrate the presence of bacilli in other sites. In 1907, however, M'Fadyean had believed that the bacilli must sometimes be carried out from the mesenteric nodes into the lungs and other organs but "apparently the conditions there are unfavourable for their growth." Jarmai (1922) recording a case of Johne's disease in a gnu (*Connochaetes albojulus*, Thom) described miliary foci in the liver from which he was able to recover *Mycobact. johnei*. Lesions of a similar nature were reported by Matthews (1930) in the livers of two affected bovines. The presence of acid-fast organisms was demonstrated with great difficulty whereas large numbers were seen in the sections from the intestines and adjacent glands. Alexejeff-Goloff (1929) further startled current opinion by reporting the isolation of Johne's bacilli from various organs and from the milk, blood and urine of cows suffering from the advanced disease. He believed that *Mycobact. johnei* often enters the circulation and invades various organs, the uterus included, and that the possibility of congenital transmission (of which he cited two possible cases) should be borne in mind. Houthuis (1932) disagreed with Alexejeff's conclusions but he reported that on very rare occasions acid-fast bacilli are found in organs other than the intestines or mesenteric lymph glands, e.g., liver, lungs and portal lymphatic glands, but these bacilli did not appear to influence in any way the progress of the disease. Hallman and Witter (1933) observed suggestive focal lesions in the livers of some cattle from a heavily infected herd, but were unable to associate them with the presence of Johne's bacilli. Dunkin (1938) has recorded cases of the disease in which the anterior skeletal glands (pharyngeal, submaxillary) were the only ones from which he could secure cultures. He considered it probable that, contrary to orthodox belief, the intestinal wall and adjacent glands are the last places to be invaded. He had already reported (1935) what may probably have been a congenital case in a calf. Borodenok (1941) examined 30 affected cows and found lesions in the gall-bladder, portal lymph glands and spleen of two of them. On the other hand Dunkin, Glover and Minett (1941) who examined 104 cattle, many

of which were clinically affected, isolated only one possible strain of Johne's bacillus from an unusual site (iliac node).

At the beginning of experiments on goats, it was thought desirable that special attention should be paid to the problems of the distribution of lesions and of the whereabouts of the bacilli in the body of infected animals, on account of its bearing on some aspects of the pathogenesis of the disease about which little is yet known. Goats that died or were killed at various intervals after infection were studied at *post-mortem* both bacteriologically and histologically as described in the section on methods. I am greatly indebted to my colleague, Dr. E. G. White, for interpreting the histological changes.

RESULTS

The results obtained in the several experiments are summarised in Table VIII in so far as they relate to (1) clinical cases ; (2) non-clinical cases ; (3) non-clinical cases, included under (2) but examined within six months after infection.

Table VIII shows at a glance that *Mycobact. johnei* was recovered from other sites in addition to the intestines and adjacent glands. This was frequently the case in clinically affected animals, the bacilli being present in the tonsils, pharyngeal, submaxillary, bronchial and mediastinal, and the portal lymphatic glands. The liver in two goats (33, 35) and the bile in one (33) also proved to be infected on culture. In contrast, animals examined within six months of infection yielded the organism from unusual foci only in a relatively small number of cases.

Failure to isolate the bacilli from some goats early after infection can be ascribed partly to the limitations of the methods used and partly to the difficulty of finding lesions of Johne's disease in those animals included in Group (3) of Table VIII which died of inter-current infection.

The problem of detecting a small number of bacilli in the large mass of mesenteric nodes or in the expanse of intestinal mucous membrane need not be stressed. As it was impracticable as a rule to deal with more than representative samples from these situations, the possibility of obtaining false negative results was much greater than in the case of other organs (tonsils, pharyngeal nodes, etc.), which could be examined as a whole. It is significant therefore that in all the early cases (Table VIII, Group 3) from which Johne's bacilli were recovered, the organs which proved most commonly affected were, in fact, the intestines and mesenteric lymphatic glands. In one animal only (52) were the bacilli not isolated from these organs when they proved to be present elsewhere.

The presence of *Mycobact. johnei* in "unusual" sites was accompanied not infrequently by typical cellular changes in which numerous bacilli were sometimes present and were, probably, the outcome of active multiplication (see Plate II, Figs. 7 and 8). These lesions,

however, did not appear to influence to any marked degree the course of the experimental disease.

The mode of infection of the glands of the head is not definitely known. It is likely that, at least, in some cases the bacilli break into the lymphatic channels of the mouth and pharynx and are conveyed to the submaxillary (mandibular) and/or the pharyngeal (medial and lateral) nodes. The tonsils with their open crypts may constitute one portal of entry from which afferent vessels reach the terminal pharyngeal lymphatic glands. It is probable that the latter do not stop all the bacilli reaching them and that some could get to the lungs and general circulation *via* the main lymphatic tract of the neck—the tracheal duct, and the anterior vena cava. The findings in goats do not suggest, however, that that is the primary route by which the intestines become infected. If the tonsils and lymphatic glands of the head were the first organs to be infected one would expect in them more frequent and more advanced lesions than in the mesenteric nodes or intestines. From Table VIII and from the discussion which follows it appears, however, that that was not the case. It is probable that the intestines are invaded directly from the lumen by bacilli which are then conveyed to the mesenteric lymphatic glands. While the factors concerned in the penetration of the intestinal epithelium are still the subject of much speculation (Hallman and Witter, 1933), experiments with acid-fast organisms other than *Mycobact. johnei* (*M. phlei*, *M. tuberculosis*, B.B.G.) have established the fact that the intestinal epithelium in various species is directly permeable and that this permeability is more intense and more regular in very young animals than in older ones (*e.g.*, age as factor of susceptibility in Johne's disease). Dunkin's assertion that in some cattle which reacted to the johnin test, the nodes of the head were the only sites from which he could recover cultures cannot be taken as evidence that the intestines and mesenteric glands are the last organs to be invaded in Johne's disease. From our knowledge of the distribution of lesions in tuberculosis, is it not possible to visualise that in some animals Johne's bacilli could localise in the glands of head and tonsils only, in some in the intestines and adjacent glands and in others especially clinical cases, in the organs already mentioned, as well as elsewhere? There is no reason to believe that during the incubation period, Johne's bacilli multiply to any great extent elsewhere than in the intestines and adjacent glands (see Table VIII). The liver, lung and/or the associated lymphatic glands of those goats which were examined within six months after infection rarely contained the specific bacilli. The data here reported do not suggest that a secondary invasion of the intestines, in the sense of Orskov, Jensen and Kobayashi (1928), is a common occurrence. These authors, studying mouse typhoid, noted that after the administration per os of *Salmonella typhi-murium*, the organisms could be detected in the faeces only for a very short time. By killing their infected mice

at intervals, Orskov *et al.*, found that after this preliminary stage, the bacilli appear to be carried mainly into the mesenteric glands, the liver and spleen where they progressively increase in numbers. The causal organism then succeed in breaking into the blood stream and a secondary invasion of the intestine, principally *via* the gall-bladder and bile duct occurs.

No attempts were made in these studies on goats to determine whether or not the bacilli enter the blood stream but such a possibility is likely and cannot be excluded. The reports of Alexejeff-Goloff (1929) and Borodenok (1941), already mentioned, as well as the isolation by the writer of a strain of Johne's bacilli from the spleens of a clinically affected goat and a clinically affected cow, suggest that bacteraemia may occur. The frequency with which the bacilli were found in the pharyngeal nodes of clinically affected animals (see Table VIII) may have been due in part to direct penetration of the bacilli through the lymphatics of the mouth and pharynx and possibly in part also to bacteraemia. Further work, however, is necessary in order to assess the importance of blood spread, especially as regards bacillary excretion through the udder and placenta.

In the literature already cited there were suggestions that in Johne's disease of cattle as in goats the causal organism may be present in "unusual" sites such as liver, lung, spleen, gall-bladder, portal lymphatic glands, tonsils and lymphatic nodes of the head. The writer has confirmed these reports by isolating *Mycobact. johnei* from the pharyngeal nodes (2 cases), the tonsils (1 case) and the spleen (1 case) of naturally affected cows in each of which the organisms were also found in the orthodox sites, intestines and mesenteric nodes.

GENERAL SUMMARY AND CONCLUSIONS

Johne's disease was set up in young goats by feeding Johne's bacilli in the form of intestinal scrapings from affected cattle or, more conveniently, with pure cultures of the organism.

A large percentage of goats so fed developed the infection in a progressive and a fatal form closely resembling that observed in naturally affected cattle.

The reactions of the infected and in-contact control goats to successive double-intradermal johnin tests followed several patterns. With increased resistance, allergy appeared to wane or disappear. Anergy in those goats dying of the experimental disease was not common. In one experiment dealing with adult goats, the allergic response was very irregular.

Avian tuberculin was inferior to johnin as a diagnostic agent in experimentally infected goats.

It was definitely established that Johne's bacilli were eliminated in the faeces not only during the clinical stages of the disease but also during the period of incubation.

The lesions in goats were similar in nature to those observed in cattle. In nine out of 18 clinically affected goats, however, caseous or calcified nodules were present in the mesenteric nodes.

Johne's bacilli were isolated not only from the intestines and mesenteric nodes but also from other organs.

The distribution of *Mycobact. johnei* in goats killed at intervals after infection suggests that the main portal of entry of the organism is the intestine.

Goats can be used in lieu of calves for the study of some aspects of Johne's disease still requiring attention.

ACKNOWLEDGMENTS

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PLATE I.



FIG. 1.—Jejunum infiltrated with epithelioid cells and lymphocytes. Haematoxylin-eosin. $\times 84$.

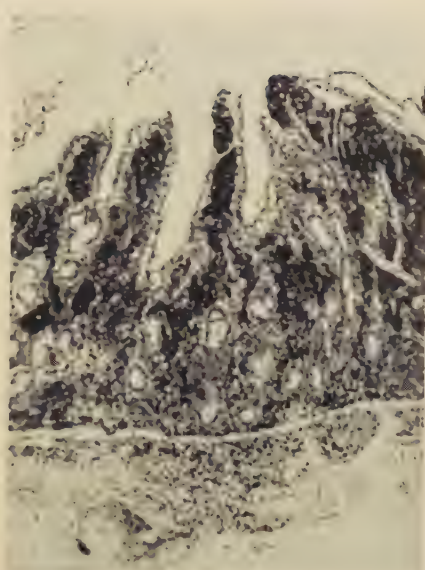


FIG. 2.—Jejunum; dark areas represent masses of bacilli. Z.N. stain. $\times 60$.

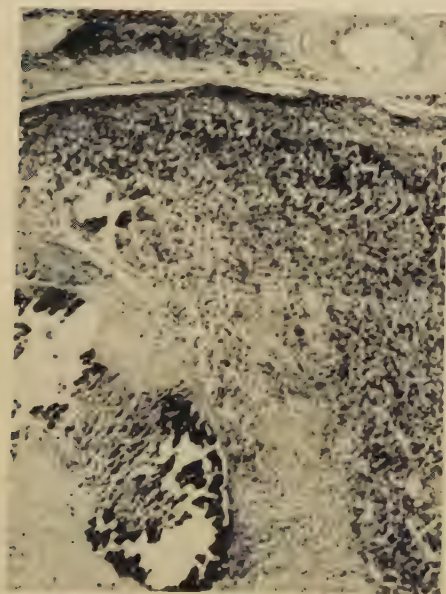


FIG. 3.—Mesenteric node; structure very altered; calcified nodule in bottom left corner. $\times 50$.



FIG. 4.—Mesenteric node showing caseous foci, masses of epithelioid cells, fibrosis. Note lymphatic vessel in capsule thickened and filled with epithelioid cells. $\times 50$.

PLATE II.

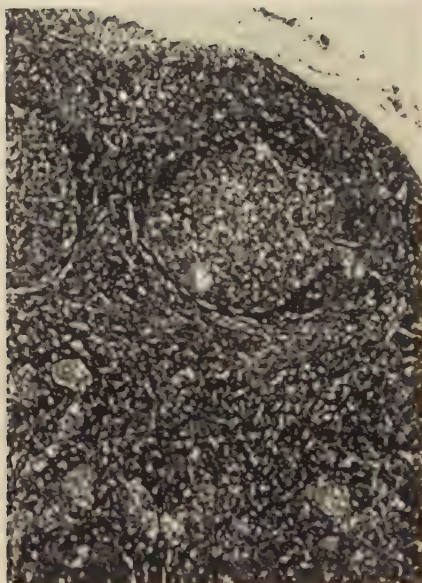


FIG. 5.—Mesenteric node showing small islets of epithelioid cells. $\times 62$.



FIG. 6.—Mesenteric node of Goat 9. Tubercles associated with the avian type of *Mycob. tuberculosis*. $\times 120$.

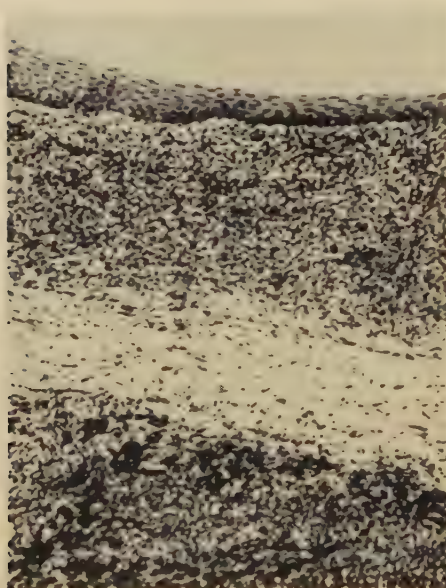


FIG. 7.—Tonsil of Goat No. 33. Dark areas are masses of Johne's bacilli. $\times 120$.

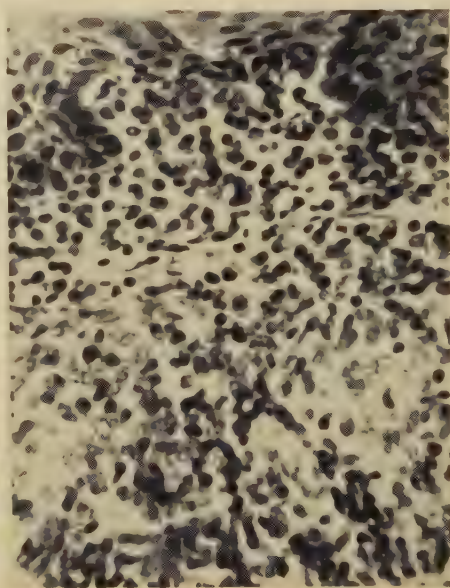


FIG. 8.—Tonsil of Goat No. 9 showing infiltration with epithelioid cells. $\times 420$.

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RINDERPEST IN SOME AFRICAN WILD MAMMALS

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INTRODUCTION

It has long been recognised that certain wild species play an important part in the epizootiology of rinderpest. This belief is the outcome of observations in field outbreaks and little by the way of controlled experiments has been done. Carmichael (1938) has given a resumé of the evidence and conclusions drawn by several workers, together with his own experiences. Several wild species are known to be susceptible to the disease and the degree of susceptibility appears to vary from species to species.

Buffalo, according to Carmichael (*loc. cit.*) and other observers are the most important of these wild disseminators of the disease, but eland also are very susceptible and play a part in its transmission to domestic cattle. Wildebeest, although formerly believed to be insusceptible, were shown by Reid (1933) and Cornell (1933) to be capable of contracting and dying from the disease. On the other hand, certain ruminants such as Coke's hartebeest show either a complete immunity or a high resistance to rinderpest. (Cornell, *loc. cit.*).

Thus, in the development of control measures, an understanding of the part played by wild animals in the dissemination of the disease is essential. Factors to be determined in relation to a wild species are its susceptibility, its degree of resistance, its ability to transmit the disease and its habits, whether gregarious, nomadic or solitary. Such knowledge in regard to many wild species is scanty and, while in some a very good idea of the position is possessed, the evidence is rarely as conclusive as is desirable.

Among the problems confronting a worker on this subject are those concerned with (a) the procuring of wild animals (b) their maintenance in satisfactory surroundings, *e.g.*, housing, feeding and management and (c) the actual virus experiments in which care must be taken to eliminate confusion which may result from intercurrent diseases, dietetic derangements and unnatural surroundings.

METHODS

The following is an account of experiments carried out on certain species of African wild fauna.

Some of the animals used were captured in the "bush" and transported to the Laboratory, some were obtained from the Sleeping Sickness Laboratory at Tinde, and some were bred in captivity.

It is not proposed to deal fully with the subject of maintenance of such animals in this article other than to say that at the time of the experiments they were thoroughly accustomed to being handled, to having their temperatures taken each morning and to the conditions under which they were maintained. All were healthy and vigorously active. Although too few animals were used to allow of fully comprehensive investigations, the results are instructive and should act as a fairly reliable guide to the knowledge required and to future work on the subject.

THE INTRODUCTION OF ACTIVE FIELD RINDERPEST VIRUS INTO A REEDBUCK (*Redunca redunca*) AND ITS PASSAGE INTO BOVINES AND PORCUPINES (*Hystrix galeata*)

An adult female reedbuck was isolated and daily temperature records were kept for a month. The average temperature over the last ten days of this period was 99° F. Representative blood smears were examined and showed no parasites nor recognisable abnormalities. Blood (5 c.c.) from an ox reacting to a virulent strain of rinderpest virus (a strain used for the production of inactivated tissue vaccine at this laboratory) were injected subcutaneously into the reedbuck. A thermal reaction developed, commencing on the third day and attaining a peak of 103·8° F. on the fifth and sixth days. On the fifth day blood slides were examined for parasites but none were demonstrable. On the sixth day 5 c.c. of blood from the reedbuck were injected into each of two oxen (Nos. 70 and 71) taken from a herd of rinderpest-susceptible cattle used for vaccine production. A further 3 c.c. of the reedbuck's blood were injected into each of two porcupines (D and E). These porcupines, together with the others used in the experiment, had been subjected to daily temperature recording for ten days previously and their bloods had been examined microscopically and found to contain no parasites. On the same day two other porcupines (B and C) were injected subcutaneously with 3 c.c. each of virulent ox blood. The average temperatures of these animals before injection, was 100·6° F. but fluctuation occurred through a range of 2 to 3 degrees. Their subsequent histories are described later, together with those of oxen Nos. 70 and 71.

The reedbuck died fourteen days after the injection of the virus. For several days before death it refused to eat, was dull and its coat was "staring." Some inflammation of the gums was noticed. Its temperature rose to 103° F. on the day before death.

Post-mortem examination revealed the following changes. The subcutaneous tissues were suffused with dark coloured blood and the superficial glands were congested. The gums showed typical rinderpest ulcers, especially near the incisors; the nostrils were inflamed; the left conjunctiva was catarrhal while the right was normal. The under surface of the tongue around its

base and on the frenum was ulcerated; the pharynx was severely inflamed and eroded. The oesophagus showed typical rinderpest ulcers throughout its length and the mucous membrane of the rumen was slightly congested. The abomasal lining was acutely inflamed in the fundus and slightly injected near the pylorus. The small intestine showed sub-acute inflammation; the caecum was heavily inflamed at the blind end and around the ileo-caecal valve, while there was medium intensity of inflammation in the colon and rectum. The pleural cavities contained a slight excess of fluid; the larynx and trachea were markedly injected and the lungs very congested. The pericardium was congested, the epicardium and myocardium were normal in appearance but the endocardium showed ecchymoses, especially about the bases of the papillary muscles and in both atria. The liver was enlarged, congested and petechiated; the gall-bladder was distended with golden-yellow bile and the blood vessels were enlarged, although the mucous membrane of the viscus was normal in appearance. The spleen was dark red and the trabeculae prominent. The suprarenal glands, the mesentery and the mesenteric, splenic and other abdominal lymph glands were congested.

The kidneys were congested and petechiated in both medulla and cortex and the capsule was adherent. The mucous membrane of the urinary bladder was inflamed. There was no reason to doubt that the animal had died of rinderpest.

The subsequent histories of the test animals inoculated from the reedbuck are as follows.

Ox No. 70 showed a thermal reaction which attained a peak of 104.8° F. on the third day and subsided by the eighth day. The clinical signs were typical of the syndrome caused by the original virus but they were somewhat milder and had abated by the eighth day.

Ox No. 71 also developed a thermal reaction commencing on the third day and reaching a peak of 105.8° F. on the fourth. The syndrome was the same as in No. 70 and had abated by the ninth day. Both these cattle were given a test dose of virulent bovine blood one month after the original injection. No. 70 showed no reaction and No. 71 developed a rise of temperature to 104° F. at 18 hours, which had subsided by the next morning. It is unlikely that this represented a reaction to rinderpest virus as there were no clinical manifestations nor was there further reaction. The blood of both test animals was examined repeatedly throughout the experiment and no parasites were found.

Porcupines D., E., B. and C. In none of these animals was there a rise in temperature or departure from health.

Blood (5 c.c.) taken from porcupine E. seven days after the injection of reedbuck blood was injected into ox No. 73. Neither thermal nor clinical reaction occurred and the ox subsequently reacted to a test dose of virulent ox blood. Similarly, blood from

porcupine B., taken on the seventh day after injection of virulent ox blood, was injected into ox No. 72. Again no reaction resulted and the ox was proved susceptible to rinderpest by a subsequent challenge dose of virus.

Thus blood from the reacting reedbuck and also virulent ox blood had no noticeable effect when injected into porcupines. Apparently the virus was killed in the porcupines as their blood subsequently failed to infect susceptible oxen. Whether this immunity was natural or acquired is unknown, but an attempt was made to clarify this question by a further experiment.

Attempt to passage rinderpest virus to young porcupines

Four young porcupines were bred in captivity and therefore were of known history. These animals were of ages ranging from one to two and a half months and were still unweaned. A daily record was kept of their rectal temperatures for 11 days, after which each received a subcutaneous injection of 1 c.c. of virulent ox blood. Temperature recording was continued and the temperatures were plotted. (See Table I.). A small rise in temperature occurred in each porcupine but in only one case (No. 3) did this resemble a rinderpest virus reaction. None of the porcupines showed any departure from health.

On the sixth day after injection, blood was drawn from each of the porcupines and 1 c.c. was injected into each of four susceptible oxen respectively. None of these reacted but all proved susceptible to a test dose of virus injected 15 days later. (See Table II.).

Except in one case the rise in temperature did not resemble a rinderpest reaction and in all cases, apparently, normal health was maintained. The rise was possibly due to the breakdown of the foreign proteins injected. Blood from the porcupines on the sixth day after the injection of virulent ox blood failed to infect susceptible oxen. It is evident, therefore, that the porcupines were highly resistant if not absolutely immune to rinderpest and, as they had not been in contact with the disease since their birth, that the immunity was natural.

THE EFFECT OF RINDERPEST VIRUS IN THE BUSH PIG (*Potamocoerus spp*)

Five bush pigs, all from the Lake Province of Tanganyika, were used. They were housed in cattle stalls at the Laboratory and maintained on a diet of cassava, groundnut, skim milk and a green fodder consisting mainly of freshly cut lucerne. This was supplemented by bone meal, common salt and small quantities of red-palm oil. The diet appeared to be satisfactory as the animals thrived in captivity. When they had become accustomed to the surroundings, daily temperature recording was commenced. At first, very erratic temperatures were noted, due no doubt to their struggling when restrained, but after a few weeks they became accustomed to handling and the temperatures became less erratic, averaging

101.4° F. At this point the experimental work was commenced. Throughout the whole experiment, blood slides were examined at frequent intervals and at no time were parasites or other abnormalities noted.

Injection of virus

Two of the pigs (C. and E.), which were about quarter grown, were inoculated with 3 c.c. of blood from an ox reacting to the rinderpest virus used for vaccine production. Thermal reactions of 104° F. and 105.2° F. respectively were recorded on the third day after inoculation. During the following two days the temperatures fell but rose again to a second peak on the seventh day after injection. Thereafter, the temperatures subsided for some days, but attained another, but lower peak, on the 17th and 18th days.

They then became normal and, after a further six days, both pigs were subjected to a large test dose of virulent ox blood, followed by a second challenge ten days later. Neither pig showed any thermal or clinical reaction to these injections. It appears, therefore, that the animals had actually suffered an attack of rinderpest as a result of the injection of virulent ox blood.

During the thermal reactions and the subsequent defervescence, both pigs showed clinical symptoms of inappetence, general malaise, as indicated by lethargy and burrowing into bedding, and diarrhoea and tenderness of the mouth. Recovery was complete and uneventful.

Contact experiment

Another bush pig (A), also about quarter grown, was put in the same stall as pigs C. and E. at the height of their reaction to rinderpest virus, *i.e.*, on the third day after injection. This pig developed a rise in temperature which attained a peak of 105° F. four days after introduction into the stall. A slight remission then occurred, but body temperature rose again to 105° F. five days later, after which a sudden temperature fall preceded death. Before death this pig showed similar symptoms to those of pigs C. and E.

Post-mortem examination of pig A. revealed epistaxis, rhinitis, conjunctivitis and ophthalmia. The buccal mucous membrane was petechiated and erosions of the gums below the incisors were present. The subcutis was congested, as were also the peritoneum, pericardium, pleura and diaphragm. The lymphatic glands were congested and the tongue, pharynx, larynx and trachea inflamed. The oesophagus also showed slight inflammation. The lungs, heart and spleen appeared normal. The liver was fatty and petechiated, while the kidneys showed petechiae beneath the capsule and congestion of the medulla. The mucosa of the stomach was acutely inflamed, especially in the fundus and near the pyloric sphincter. The small intestine, caecum, colon and rectum were intensely inflamed, especially near the ileo-caecal valve. There was acute inflammation of the urinary bladder.

Bush pig A. contracted rinderpest by contact with pigs C. and E. and so it appears that the disease in bush pigs is infectious. Contact in this case was very close as the animals ate and drank from the same troughs and were confined together in a small space. These creatures appear to be very gregarious and it is probable that close contact, direct and indirect occurs in the natural state.

Passage of virus in the bush pig

An adult male bush pig (D.) was inoculated with 4 c.c. of blood taken from the young pig A. during the latter's thermal reaction. On the third day after inoculation pig D. showed a thermal reaction of 103·6° F. The temperature fell subsequently but rose to a second peak of 103° F. on the sixth day. This was followed by a slow decline of temperature until the animal died, 12 days after the injection. The *post-mortem* picture in this animal was very similar to that in pig A. The main divergences from the latter were found in the liver, which appeared to be normal, the lungs, which were completely hepatised, and the gastric mucosa in which there was marked ulceration in addition to the usual inflammation.

Saline washings from the heart of bush pig D. obtained on the day of its death, together with a pulped portion of its spleen, were injected into a young adult bush pig (B.). Neither thermal nor clinical reaction resulted. Twelve days after the above injection this pig received subcutaneously 10 c.c. of virulent blood from a reacting ox. A thermal reaction which started on the third day and attained a peak of 104·6° F. on the fourth day, was followed by a remission and a subsequent peak of 104·8° F. seven days after the first, *i.e.*, on the eleventh day. After that, recovery occurred uneventfully. The clinical symptoms in this animal were similar to, but rather more severe than, those shown by the previously infected bush pigs. Dysentery which lasted for three days commenced two days after the second thermal peak. Subsequent challenge with virulent ox blood showed the animal to be immune, no reaction occurring.

Thus the virus had disappeared from the heart and spleen of the pig D. by the time of its death, nine days after the peak of the thermal reaction. The three bush pigs which survived the attack of rinderpest, *viz.*, B., C., and E., were tested with rinderpest virus 15 months later and found still to be immune.

SUMMARY

A reedbuck was infected with rinderpest virus and died after suffering from the disease. The *post-mortem* features are described.

Virulent reedbuck blood was injected into susceptible oxen and porcupines, and virulent ox blood was also injected into porcupines. The oxen developed rinderpest whereas the porcupines did not. Blood from the porcupines taken seven days after the injection of virus failed to infect susceptible oxen.

Four young porcupines bred in captivity were injected with virulent ox blood and although their temperatures rose afterwards, the reaction was not considered to be due to rinderpest. Their blood, taken six days after the injection, failed to infect susceptible oxen. Porcupines are therefore considered to be naturally immune to rinderpest.

Rinderpest virus was introduced into five bush pigs, four receiving the infection by subcutaneous injection and one by contact. All suffered severely from the disease and two died. Those which recovered proved to be immune 15 months after the attack. The clinical picture of rinderpest in bush pigs and *post-mortem* findings are described. The blood and spleen tissue of a bush pig which died of rinderpest were shown to be free of virus nine days after the peak of the thermal reaction.

Bush pigs are considered to be capable of disseminating rinderpest in the wild state.

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BRUCELLA ABORTUS INFECTION AMONG BUFFALOES IN EGYPT

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INTRODUCTION

THE importance of *Brucella abortus* infection among cattle, including buffaloes, in Egypt has not so far been fully appreciated either by the cattle owners or by the veterinary services. In the latter case, this is probably because attention has hitherto been focused mainly upon epizootic diseases, of which cattle plague remains the most striking example. Private veterinary practice among farm livestock in Egypt is very limited and isolated cases of abortion would not come to the attention of veterinary officers so frequently as to compel investigation even if they were relatively common and widespread. Moreover, in Egypt it is the buffalo which is the chief milk-producing animal, largely on account of the high butter-fat content of its milk (7 to 9 per cent.) and its suitability as a milk-producing animal in hot climates. Ordinary cattle are kept mainly to breed and rear calves destined either for beef or for work and their milk yield is low. For these reasons, in order to make a preliminary survey of the extent of *Br. abortus* infection in Egypt, it was thought the most convenient and profitable line of enquiry would be to investigate the incidence of the organism in the milk supply of which by far the larger proportion is of buffalo origin.

THE INCIDENCE OF *Brucella abortus* INFECTION IN THE MILK OF BUFFALOES

The literature on brucella infection in buffaloes is somewhat scanty considering the widespread domestication of this species in tropical and semi-tropical countries. Gabi and Cantani (1933) compared their susceptibility to *Br. abortus* and *Br. melitensis* strains; while among the bison kept in the Yellowstone National Park, U.S.A., Mohler (1917) first detected infection, which was later studied by Creech (1930), Rush (1930, 1932, 1933) and Tunnicliff and Marsh (1935). In the conditions of overcrowding within this reserve, the infection reached high proportions. In India, where buffaloes are kept in much the same conditions as in Egypt, the incidence of brucella infection does not seem to be as high as in some of the ordinary cattle such as those of the Government military dairy farms (Edwards, 1925), though Priestley (1946), working at the

Some of this matter formed part of a thesis presented to the School of Veterinary Medicine, Fouad I University, for the degree of M.V.Sc. in April, 1943.

Indian military veterinary laboratory, Lahore, has recently claimed that it is probably very prevalent.

EXPERIMENTAL

Two hundred individual samples of milk were collected from buffaloes kept in different dairies in Cairo and district. The samples, which comprised the final strippings, were drawn directly from the teat into a sterile 100 c.c. flask, care being taken to avoid extraneous contamination by thoroughly washing the udder and the milker's hands. They were immediately taken to the laboratory, where each was examined for brucella infection by direct culture, by the agglutination test on the milk whey, and by animal inoculation.

Direct culture method.—Samples were examined as quickly as possible after collection in order to eliminate the risk that acid-forming and other rapidly multiplying bacteria might kill brucella organisms. On two occasions, when the samples had to be kept overnight, 1 per cent. boric acid was added to the milk to prevent the multiplication of contaminants (Gill, 1932; Traum and Henry, 1930; Stockmayer¹ 1935; Huddleston, 1934).

Each milk sample (50 c.c.) was centrifuged for 30 minutes at 3,000 r.p.m. in two sterile centrifuge tubes. The whole sediment of the two tubes and part of the cream layer were suspended in 10 c.c. sterile saline. A few drops were spread on the surface of each of four plates of liver infusion agar to which gentian violet was added in a dilution of 1 : 200,000.

Two plates were incubated aerobically and the other two in a jar containing 10 per cent. CO₂. The method of incubating all the plates aerobically for 15 to 24 hours and then transferring them to the CO₂ jar as recommended by Huddleson was found to be not only unnecessary but troublesome in that it encouraged the growth of contaminants, especially moulds, which made subsequent growth of brucella impossible. The CO₂ atmosphere, although it does not entirely inhibit the growth of contaminants, seems to have some effect in retarding their growth. Thus preliminary aerobic incubation was discarded and the CO₂ method was alone employed using two seeded plates only.

After four days' incubation at 37° C., the plates were examined and any colony resembling that of *Br. abortus* was subcultured. Films were examined, a set of sugar media were inoculated, and finally a suspension tested by means of the agglutination test against a known positive *Br. abortus* rabbit anti-serum. If the cultural characters, staining reactions and sugar tests were positive and the suspension agglutinated with the above serum to a titre of not less than 1 : 400, the organism examined was designated as *Br. abortus*.

Agglutination test with milk whey.—M'Fadyean and Stockman (1915) originally discovered the presence of brucella agglutinins in milk. Owing to the opacity caused by adding whole milk to the culture, whey obtained by adding a few drops of rennet to the milk

and warming at 45° C. for 15 minutes to cause clotting, was used : it was tested against a suspension of *Br. abortus* containing about 1,000 million organisms per c.c. in dilutions from 1 : 10 to 1 : 80. The tubes were incubated overnight at 37° C. and then left at room temperature for another 24 hours before the results were recorded. Any sample showing complete agglutination at 1 : 20 was considered positive.

Animal inoculations.—The above 200 samples were used for animal inoculations. At first rabbits, mice, rats and guinea-pigs were employed, but later it was found that no advantage was to be obtained by using any species other than the guinea-pig.

The whole sediment, and part of the cream obtained from the centrifugation of 50 c.c., were suspended in 10 c.c. of sterile saline and injected intraperitoneally into two male guinea-pigs, each weighing between 300 and 400 g. Apart from an occasional death due to contaminating bacteria, the inoculated animals remained apparently normal and gained in weight. At the end of the fourth week, some blood was withdrawn from an ear vein and tested for brucella agglutinins. Any animal whose serum showed a titre of 1 : 50 or above was killed ; the remainder were left till the end of the sixth week. Cultures were made from the spleen and lymph glands of every animal whether those organs were showing macroscopic changes or not. It was not unusual in this work to isolate *Br. abortus* from normal-sized, healthy looking spleens, while some enlarged congested spleens proved negative on cultural examination.

RESULTS

From the 200 milk samples, *Br. abortus* was isolated from four only by direct culture. The strains isolated were tested by Huddleson's method (1934), with the following results : none grew in an ordinary atmosphere when first isolated ; all strains produced a moderate amount of H₂S for two to three days only ; all grew well on media containing 1 : 25,000 basic fuchsin but not on media containing 1 : 50,000 thionin. These findings proved the isolated organisms to be *Br. abortus*.

Indirect evidence of infection could be demonstrated by the presence of agglutinins in the whey of 16 samples (8 per cent.). The rapid agglutination test described by Riedmuller (1934) was tried on about 50 samples, but the results were not sufficiently clear to encourage its routine use. The ring test of Fleischhauer (1937) was carried out on many samples with negative results.

Direct evidence of infection was obtained in 30 samples (15 per cent.) by guinea-pig inoculation, which thus far surpassed in efficacy the other two methods. It is realised that even guinea-pig inoculation may have failed to demonstrate the organism in all infected samples. Some positive cases may have been missed on account of the organisms being present in sub-infective doses, or through being of low viability at the time of test ; a third possible cause of

error is low adaptability of the organism to guinea-pig tissue. Nevertheless, the evidence clearly indicates that no fewer than 15 per cent. of the cow-buffaloes of Cairo and district are excreting brucella in their milk. All brucella organisms found were of the *abortus* and not of the *melitensis* type. This does not exclude the possibility that some of the strains were pathogenic for man, but the custom among Egyptians of boiling all milk before consumption largely excludes risk of infection from this particular source.

The following technical details are noteworthy. Gentian violet 1:200,000, added to the liver agar was not sufficient to inhibit the growth of gram-negative contaminating bacteria and fungi, while inoculated plates should be put immediately in CO₂ jars without previous aerobic incubation. It was confirmed that guinea-pigs are the most reliable animals for diagnosing *Br. abortus* in milk and that the intraperitoneal route of inoculation is the most suitable. The sera of the guinea-pigs should be examined for agglutinins at the end of the third to fourth week after inoculation, and if any do not show a positive reaction at that stage they should be left until the sixth week before they are killed. A cultural examination of spleen or lymph glands for brucella infection is essential; macroscopic examination alone leaves many cases undetected. Lesions, when present in the spleen, took the form of greyish caseating nodules, but sometimes the only change was a mild inflammation with slight enlargement, while in a few cases no macroscopic changes could be detected. On the whole, the Cairo strains of *Br. abortus* were of relatively low pathogenicity for, but apparently high adaptability to, the guinea-pig.

THE CORRELATION BETWEEN THE SPECIFIC AGGLUTININ TITRE OF THE BLOOD SERUM OF BUFFALOES AND THE EXCRETION OF BRUCELLA IN THE MILK

Earlier workers have shown that some degree of correlation exists between the blood serum agglutinin titre and the excretion of *Br. abortus* in cow's milk, although cases have been encountered in which the organism has been isolated from the milk of animals showing a very low serum titre. Such cases, however, are rare and it has been claimed that if such animals are tested a month or so later the titre will be found to have risen much higher.

Gaiger and Davies (1932) tabulated the findings of Tulberg (1931), Gilman (1931), Klimmer (1932), Karsten (1932) and Proscholdt (1932) on this correlation. All agree that with rising agglutinin titre in the blood serum there is increased excretion of brucella in the milk, but whereas some authors appear to conclude that milks from cattle showing a low serum titre represent a negligible risk, others do not support that conclusion. Tulberg, for example, found that organisms were excreted in the milk of some cows whose titre did not exceed 1:20, while Hayes and Barger (1935) recorded brucella in the milk of a cow with a negative serum titre. Carpenter (1926)

has reported excretion in the milk of cows which had been inoculated with live vaccines but which afterwards gave very low serum titres ; in addition, Hart and Traum (1925) and Sachweh (1932) have examined the excretion in animals which have not developed agglutinins after infection.

It was in part these controversial views regarding the above relationship that led us to carry out the present work to determine the lowest blood serum titre that could be considered to denote actual excretion of brucella in the milk. It has to be recognised that excretion may be intermittent and that the negative results obtained in a single milk sample may not be repeated on further samples. For these reasons, even the detection of occasional infection in the milks of buffaloes showing very low blood serum titres cannot be held to be of negligible significance.

EXPERIMENTAL

Two hundred buffalo-cows in milk were examined at the abattoir. Firstly, 50 to 100 c.c. of milk were withdrawn from each buffalo into a sterile 100 c.c. flask, taking equal amounts from each quarter and using the same precautions as in the work cited above to exclude extraneous contamination. The animals were then slaughtered and about 10 c.c. of blood were taken from each into a sterile test-tube. On arrival at the laboratory, the milk samples were examined immediately for the presence of brucella organisms by intraperitoneal guinea-pig inoculation according to the method described above.

After separation of the serum, blood samples were subjected to the agglutination test using suspensions of both *Br. abortus* and *Br. melitensis* (10^9 organisms per c.c.). The tubes were incubated at 37° C. for 24 hours, after which they were left at room temperature for another 24 hours before the results were finally recorded. As no difference in the titre could be detected between the agglutinating power of the serum towards the two organisms, *Br. melitensis* was omitted in the second half of the work. The results are summarised in the table overleaf.

The following are the chief points of significance brought forth from comparison of the above data. First, no milk withdrawn from any buffalo which proved negative to the agglutination test upon its blood serum proved to be infected with brucella organisms. Secondly, even at the lowest agglutination titre which indicated infection, namely, 1 : 20, some of the buffaloes (6.6 per cent.) excreted brucella in their milk upon examination of a single sample only. Thirdly, with increase in the titre of the serum in agglutinins, there was a corresponding increase in the proportion of the milks discovered to be infected with brucella upon examination of a single sample.

It is clear that no buffalo which gives a positive blood serum agglutination reaction in Egypt can be declared to secrete milk which is not infected with brucella. There appears to be a correlation

between the agglutination titre of the serum and the incidence of infection of the milk as disclosed by the greater frequency of infection in milk samples derived from cow-buffaloes showing a high titre in their sera. It would be unsafe to assume that it is only those buffaloes which show a high titre in their sera that are potential sources of danger from consumption of their raw milk.

TABLE
AGGLUTINATION TESTS ON BLOOD SERA OF BUFFALOES

Highest serum titre	Number of buffaloes showing serum reactions	Number of buffaloes showing <i>Brucella</i> infection in milk
Below 1:20	125 (62.5 per cent.)	0 (0 per cent.)
1:20	30 (15 ")	2 (6.6 ")
1:40	24 (12 ")	5 (20.7 ")
1:80	15 (7.5 ")	8 (53.3 ")
1:160	6 (3 ")	5 (83.3 ")
1:320	0	—

CONCLUSIONS

Using the guinea-pig inoculation test, evidence of *Br. abortus* infection was obtained in 30 (15 per cent.) of 200 samples of milk taken from cow-buffaloes in Cairo and district.

Direct culture tests and examination of the whey for specific agglutinins proved to be far less reliable as diagnostic tests.

Tests for brucella infection on the blood sera of 200 Egyptian buffaloes (75 were infected and 125 were negative), and on the milks showed a degree of correlation between the titres of the sera in agglutinins and the intensity of infection of the milks.

It proved unsafe, however, to assume that the milk of any buffalo showing only a low positive reaction to the agglutination test upon its blood serum was necessarily free from brucella infection of the milk.

None of the 125 buffaloes showing a negative reaction to the blood serum test, was found to excrete brucella organisms in the milk.

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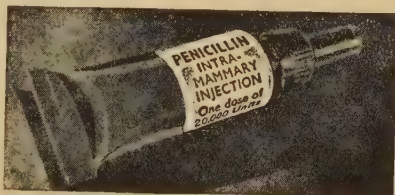


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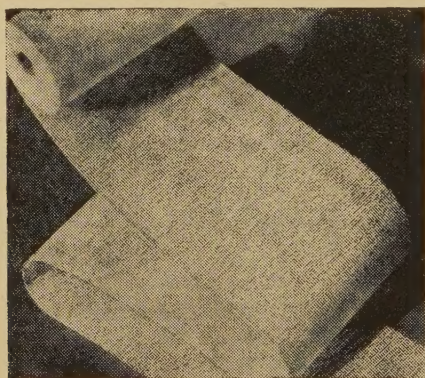
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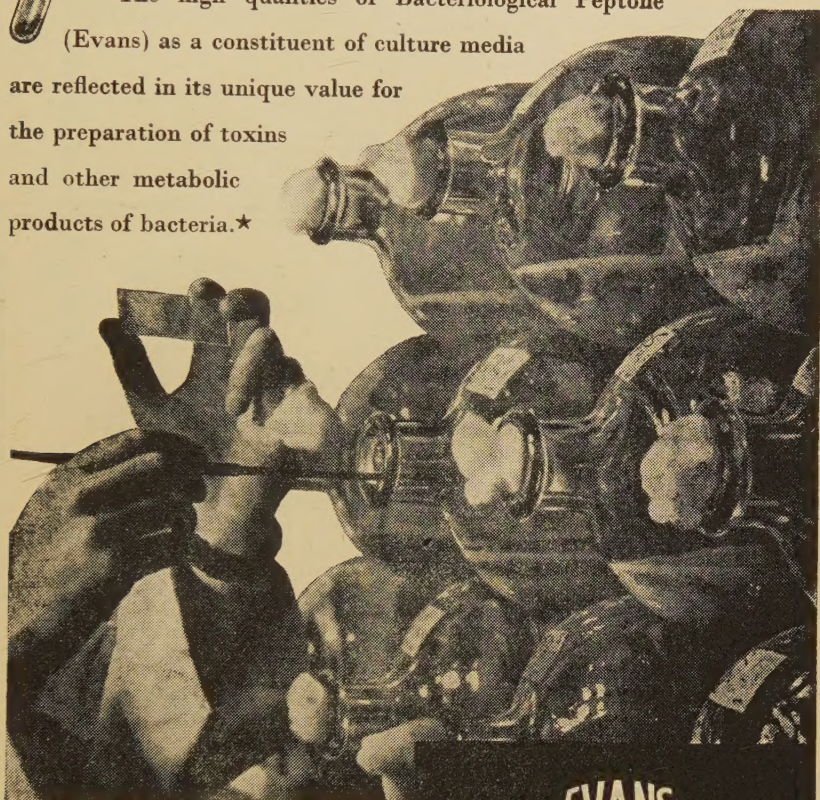
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